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HARAYAMA SHIGEAKI

(54) METHOD FOR IDENTIFYING AND SPECIFICALLY DETECTING SLOWLY GROWING MYCOBACTERIA USING CHARACTERISTIC BASE SEQUENCE PRESENT IN DNA GYRASE GENE (57)Abstract:

PROBLEM TO BE SOLVED: To allow accurately identifying/detecting slowly growing mycobacteria which can not be easily detected so far.

SOLUTION: This is a method for identifying and detecting slowly growing mycobacteria utilizing a characteristic base sequence present in gyrB gene.

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(21)出願番号 特願平11-312525 (71)出願人 591001949 株式会社海洋バイオテクノロジー研究所 (22)出顧日 平成11年11月2日(1999.11.2) 東京都文京区本郷1丁目28番10号 (72)発明者 笠井 宏朗 岩手具釜石市平田第 3 地割75番 1 株式会 社海洋パイオテクノロジー研究所釜石研究 所内 (72)発明者 江崎 孝行 岐阜県岐阜市司町40番地 岐阜大学医学部 内 (74)代理人 100091096 弁理士 平木 祐輔 (外2名)

(54) 【発明の名称】 DNAジャイレース遺伝子中に存在する特徴的な塩基配列を用いた遅発育性マイコパクテリアの 同定法及び特異的検出法

(57)【要約】

【解決手段】 gyrt遊伝子中に存在する特徴的な塩基配列を利用して遅発育性マイコバクテリアの同定及び検出を行う方法。

【効果】 従来、同定及び検出が困難であった遅発育性マイコバクテリアについても正確に同定・検出を行うことが可能になる。

【特許請求の範囲】

【請求項1】 遅発育性マイコバクテリアのDNAジャイ レースβサブユニットをコードするDNA中の配列番号7. ~22に対応する領域を増幅し、その増幅断片の塩基配 列を配列番号7〜22記載の塩基配列と比較し、各配列 からの遺伝学的距離を求め、その遺伝学的距離により前 記遅発育性マイコバクテリアの同定を行うととを特徴と する遅発育性マイコバクテリアの同定方法。

【請求項2】 配列番号4記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 10 を含み、プライマー又はプローブとして実質的に機能す るオリゴヌクレオチドを、プライマー又はプローブとし で用いるととにより、マイコバクテリウム・カンサシイ **を検出することを特徴とするマイコバクテリウム・カン** サシイの検出方法。

《請求項3 】 配列番号4記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質的に機能す るオリゴヌクレオチドを含むことを特徴とするマイコバ クテリウム・カンサシイの検出用キット。

【請求項4】 配列番号6記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質低に機能す るオリゴヌクレオチドを、プライマー又はプローブとし て用いることにより、マイコバクテリウム・ガストリを 検出することを特徴とするマイコバクテリウム・ガスト

【請求項5】 配列番号6記載のアミノ酸配列の一部若 しくは全部をコードする配列、又はそれと相補的な配列 を含み、プライマー又はプローブとして実質低に機能す るオリゴヌクレオチドを含むことを特徴とするマイコバ クテリウム・ガストリの検出用キット。

【発明の詳細な説明】

[0001]

【発明の属する技術分野】本発明は、結核並びに非結核 性抗酸症の原因菌として多くの症例数のある遅発育性マ イコバクテリアのDNAジャイレースβサブユニットをコ ードするDNA(以下、「gyrB遺伝子」という)の塩基配列 を利用した同定・検出法に関するものである。本発明の 同定・検出法は、各種産業分野において有用である。

[0002]

【従来の技術】ヒトに結核並びに結核類似症を引き起こ す抗酸菌種は遅発育性マイコバクテリアの属する種が複 数知られている。その中でも臨床例としてはマイコバク テリウム・ツベルクローシス・コンブレックス (Mycoba cterium tuberculosis complex)、マイコバクテリウム ・アビウム・コンプレックス (Mycobacterium avium co mplex)、マイコパクテリウム・カンサシイ(Mycobacte rium kansasii) がその大半を占めている。最近では後

細菌が全身播種性感染を引き起こし、AIDS患者の予後の 重大な問題となっている。

【0003】従来とれらの菌種の同定・検出は、培養に 基づく生理生化学的な方法によって行われていた。例え は、遅発育性マイコバクテリアの中には、1)光を照射 した後に暗所で培養した場合にのみ黄色に発色するグル ープ(光発色菌)、2)光を照射せずに培養しても発色 するグループ(暗発色菌)、3)光を照射しても発色し ないグループ (非発色菌) の3 グループが存在するの で、この発色の違いを利用して同定・検出を行うことが 行われてきた。また、培養した細菌がカタラーゼ生産を 行うかどうか、ウレアーゼ活性、トゥイーン加水分解活 性あるいは硝酸塩還元活性を示すかどうか、などにより 同定・検出を行う方法も知られていた。

【0004】しかし、これらの検査は純粋培養を必要と する上、比較の対象となる表現型は変化しやすく、判定 が主観的になりがちであった。その結果、時間がかかる 上に、正確な種の判定は極めて困難であった。この様な 問題点を解決するために、近年になってポリメラーゼ連 鎖反応(以下、「PCR」という)法などを用いて遺伝子 の特定の塩基配列の有無を判定する同定・検出の方法が 考案され利用されるようになった。PCR法は、培養を必 要とせず迅速かつ客観的な判定を得るという点で、遅発 育性のマイコバクテリアの同定・検出に適している。 【0005】その際、用いられる遺伝子は多くの場合、 rRNA遺伝子である。T. Rogallら(1990. J. Gen. Micro biol. 136, 1915-1920) は、16S rRNA配列を用いたPCR に基づくマイコバクテリア種の同定法を提案した。しか しながら、これらのプライマーは、異なった表現型特性 を示すマイコバクテリウム・ガストリ(Mycobacterium gastri) とマイコバクテリウム・カンサシイを区別でき なかった。一方B. Boddinghausら(1990. J. Clin. Mic robiol. 28: 1751-1759) は、ヒト型結核菌グループや **鳥型結核菌-バラ結核菌およびマイコバクテリウム・イ** ントラセルラーレ (Mycobacterium intracellulare) グ ループに特異的である16S rRNA配列に由来するオリゴヌ クレオチドを報告した。とのオリゴヌクレオチドを用い ても種のレベルでの同定を行う為に必要な解像度は得ら れなかった。これらのrRNA遺伝子配列を利用した同定法 40 は、現在商品化され、日本ロッシュから「アンプリコア ・マイコバクテリウム」という商品名の遺伝子診断キッ トとして販売されている。その他にも、東洋紡社 (特開 平10-323189号公報) やベクトン・ディッキンソン・ア ンド・カンパニー(特開平10-057098号公報)から rRNA 配列を利用した検出あるいは同定法が開示されている。 上に記したような二つの種を判別できない問題を解決す るために、16S rRNAと23S rRNAの間の領域の配列を用い た同定・検出法がA. Rothらによって提案されている(1 998. J. Clin. Microbiol. 36: 139-147)。しかし、16 天性免疫不全症候群 (AIDS) 患者等においてはこれらの 50 S rRNAと23S rRNAの間の領域は約200塩基対しかなく、

とのような短い配列により精度の高い分子系統学的解析 は困難で二菌種のどちらの配列とも一致しない中間的な 配列を持つ新規株が現れた場合、どちらにより近縁かと いった判断を下すことができない一方、シュードモナス (Pseudomonas) 属、アシネトバクター (Acinetobacte r) 属を始め、多くの細菌で、進化速度の速いタンパク 質をコードする遺伝子、なかでもgyrB遺伝子の1200塩基 対の配列を用いることによって、より詳細で正確な分類 ・同定ができることが示された (Yamamoto, S. and S. -1109. Yamamoto, S. and S. Harayama. 1996. Int. J. Syst. Bacteriol. 46: 506-511. Harayama, S. and S. Yamamoto. 1996. p250-258 In Molecular Biology of Pseudomonas T. Nakazawa, K. Fukuda, D. Haas, S. Si Iver (eds) ASM press, Washington, D.C., 山本 敏、 原山重明、化学と生物 1996 第34巻 第3号 p. 149-15 1. 山本 敏、原山重明、農芸化学会誌 1997 第71巻 第9号 p.894-897.)。

【0006】gyrB遺伝子以外のタンパク質をコードする 遺伝子を用いて遅発育性マイコバクテリアの同定を行う 試みは行われている。例えば、C. T. Shivannvarらはス ーバーオキシド・ディスムターゼ遺伝子を利用して遅発 育性のマイコバクテリアの系統関係と抗原性の関係を論 じ (1994. J. Clin. Microbiol. 32: 2801-2812)、D. 5. Swansonらは65 kD 熱ショックタンパク質遺伝子を用 いて鳥型結核菌ーパラ結核菌およびマイコバクテリウム ・イントラセルラーレグループの詳細な分類を試みた (1997. Int. J. Syst. Bacteriol. 47: 414-419)。米 国アボット・ラボラトリーズは特表平10-500567 (国際 公開番号W095/31571) としてrRNA遺伝子以外にもマイコ 30 バクテリウム・ツベルクローシスのタンバク質抗原Bを コードする遺伝子や、マイコバクテリウム・ツベルクロ ーシスの65 kD熱ショックタンパク質、10kD熱ショック タンパク質等の遺伝子配列並びに挿入配列IS987やIS611 0の関連する配列を用いた検出法を開示している。他に もベクトン・ディッキンソン・アンド・カンパニーはマ イコバクテリウム・パラツベルクロシスの70kg熱ショッ クタンパク質をコードする遺伝子に由来する検出・同定 するプローブを特開平06-319560として開示している。 しかしながら、これらの遺伝子のうち、分子系統学的に 40 得られるデーターと従来の分類学的な手法による種の同 定と比較した場合、矛盾が認められないことが示されて いるのはgyrB遺伝子のみであり(Yamamoto and Harayam a 1998. Int. J. Syst. Bacteriol. 48: 813-819. Yama moto et.al. 1999. Int. J. Syst. Bacteriol. 49: 87-95. Suzuki et al. 1999. Int.J. Syst. Bacteriol. in press, Kasai et al. 1999. Int. J. Syst. Bacterio 1. in press)、新規に分離された株についても正確な 分子系統学的な位置を決定でき、更に他の遺伝子では区 別できなかった近縁種の区別できることなどから他の遺 50 テリウム・カンサシイの検出方法、及び前記オリゴヌク

伝子を用いた方法より有効な方法である。

[0007]

【発明が解決しようとする課題】gyrt遺伝子を用いた細 菌の同定・検出法については、既に本出願人により出願 されている(特開平11-169175号公報)。しかし、この 公報中では、遅発育性マイコバクテリアの同定・検出を 行うに当たってgyrB遺伝子中のどの領域を利用すればよ いかについては明らかにされていなかった。

)

【0008】遅発育性マイコバクテリアには結核などの Harayama. 1995. Appl. Environ. Microbiol. 61: 1104 10 重要な疾病の原因細菌が含まれるため、この細菌群を正 確に同定・検出するための方法が強く望まれている。一 方、遅発育性マイコパクテリアは、生育速度が通常の細 菌よりも遅いため、細菌の培養を必須とする生理生化学 的方法では同定・検出が難しい。本発明は、このような 技術的背景の下になされたものであり、gyrB遺伝子を利 用した遅発育性マイコバクテリアの同定・検出方法を提 供することにある。

[0009]

【課題を解決するための手段】本発明者らは遅発育性マ 20 イコバクテリアの標準株のgyr8遺伝子配列を決定した。 それらの配列に対して臨床から分離された株の分類学的 な位置づけを行った。分類学的位置づけが矛盾のないと とを、細菌の種の同定の標準的方法であるDNA-DNA 交雑 法によって確認した。

【0010】また、遺伝子配列を利用した細菌の検出法 として最も一般的に用いられる16SrRNA遺伝子の配列で は区別できない非結核性抗酸菌マイコバクテリウム・ガ ストリとマイコバクテリウム・カンサシイの標準株のDN AからgyrB 断片をPCR法によって増幅し塩基配列を決定 した。得られた配列を比較したところ、両株の16S rRNA 遺伝子配列は同一であるにも関わらず、両株のqyr8遺伝 子配列1257塩基配列中66箇所が異なっていることを見い 出した(図1)。それらの配列の違いを利用して両菌種 それぞれに特異的なPCR増幅を可能にしたブライマーを 設計した。

【0011】本発明は、以上の知見により完成されたも のである。即ち、本発明は、遅発育性マイコバクテリア のgyrB中の配列番号7~22に対応する領域を増幅し、 その増幅断片の塩基配列を配列番号7~22記載の塩基 配列と比較し、各配列からの遺伝学的距離を求め、その 遺伝学的距離により前記遅発育性マイコバクテリアの同 定を行うことを特徴とする遅発育性マイコバクテリアの 同定方法である。

【0012】また、本発明は、配列番号4記載のアミノ 酸配列の一部若しくは全部をコードする配列、又はそれ と相補的な配列を含み、プライマー又はプローブとして 実質的に機能するオリゴヌクレオチドを、ブライマー又 はプローブとして用いることにより、マイコバクテリウ ム・カンサシイを検出することを特徴とするマイコバク

レオチドを含むマイコバクテリウム・カンサシイの検出 用キットである。

【0013】更に、本発明は、配列番号6記載のアミノ 酸配列の一部若しくは全部をコードする配列、又はそれ と相補的な配列を含み、ブライマー又はブローブとして 実質低に機能するオリゴヌクレオチドを、ブライマー又 はプローブとして用いることにより、マイコバクテリウ ム・ガストリを検出することを特徴とするマイコバクテ リウム・ガストリの検出方法、及び前記オリゴヌクレオ チドを含むマイコバクテリウム・ガストリの検出用キッ 10 配列番号40により表されるプライマーを例示することが

[0014]

【発明の実施の形態】以下、本発明を詳細に説明する。

(1)同定方法

本発明の遅発育性マイコバクテリアの同定方法は、遅発 育性マイコバクテリアのgyrB中の配列番号7~22に対* *応する領域をPCRにより増幅し、その増幅断片の塩基配 列を配列番号7~22記載の塩基配列と比較し、各配列 からの遺伝学的距離を求め、その遺伝学的距離により前 記遅発育性マイコバクテリアの同定を行うことを特徴と する。

【0015】本発明において同定とは、分子系統学的な 手法等によって細菌の分類学的な位置を定めることをい う。gyrB中の配列番号7~22に対応する領域を増幅す るためのブライマーとしては、例えば、配列番号39及び できるが、とれらに限定されるわけではない。配列番号 - 7~22記載の塩基配列と、対応するアミノ酸配列及び 由来とする微生物の名称との関係は、下表の通りであ る。

[0016]

【表1]

		1 2 1 2 1 2	4.4 C.X X (表]]
	塩基配列	アミノ酸配列	由来とする微生物の名称
	配列番号7	配列番号23	マイフバクラリナン
	配列番号8	配列番号24	マイコバクテリウム・シミエ
	配列番号9	配列番号25	マイコバクテリウム・ボビス
•	配列番号10	配列番号26	マイコバクテリウム・スツルガイ
	配列番号11	I	マイコバクテリウム・マルモエンセ
	配列番号12	I	マイコバクテリウム・イントラセルラーレ
	配列番号13	1000	マイ コハクアリウム・アピウム
- [配列番号14	******	マイコバクテリウム・ゴルドナエ
	配列番号15		アミコパクテリウム・アフリカヌム
- 1	配列番号16		マイコパクテリウム・ツベルクローシス
- [配列番号 1.7	四月五月日	マイコバクテリウム・ガストリ
	配列番号18	配列番号3-3	マイコバクテリウム・マリヌム
		配列番号34	マイコバクテリウム・ミクロティ
		即列母方35	マイコバクテリウム・アジァティチュ
		200	マイ コパクテリウム・スクロファラル ウューー
			マイコパクテリウム・プランデリ
L	配列番号22	配列番号38	マイコバクテリウム・パラツベルクロシス
			ニンニンノンベルクロジス

【0017】遺伝学的距離は、例えば、Phylip プログ ラムの説明書にあるFelsenstein記載の方法に従って求 めることができる(Felsenstein, J. 1993. PHYLIP(Phy logenyInference Package)version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle, U.S.A.).

【0018】(2)特異的検出

本発明のマイコバクテリウム・カンサシイの検出方法 は、配列番号4記載のアミノ酸配列の一部若しくは全部 をコードする配列、又はそれと相補的な配列を含み、プ ライマー又はプローブとして実質的に機能するオリゴヌ クレオチドを、プライマー又はブローブとして用いると とを特徴とするものである。また、本発明のマイコバク テリウム・カンサシイの検出用キットは、前記オリゴヌ クレオチドを含むことを特徴とするものである。

【0019】本発明のマイコバクテリウム・ガストリの

くは全部をコードする配列、又はそれと相補的な配列を 含み、ブライマー又はプローブとして実質低に機能する オリゴヌクレオチドを、ブライマー又はプローブとして 用いるととを特徴とするものである。また、本発明のマ コバクテリウム・ガストリの検出用キットは、前記オリ ゴヌクレオチドを含むことを特徴とするものである。

【0.020】ととで、「プライマー又はプローブとして 実質的に機能する」とは、特異的なアニール又はハイブ リダイズが可能な程度の長さを有するという意味であ り、検出対象とするDNAとアニール又はハイブリダイズ する配列を有しているが、その長さが短いため非特異的 なアニール又はハイブリダイゼーションを頻繁に起こ し、特異的な検出に使用できないようなオリゴヌクレオ チドを排除する趣旨である。

【0021】マイコバクテリウム・カンサシイの検出に 利用できるオリゴヌクレオチドとしては、配列番号3に 検出方法は、配列番号6記載のアミノ酸配列の一部若し 50 より表されるオリゴヌクレオチドを例示でき、マイコバ

クテリウム・ガストリの検出に利用できるオリゴヌクレオチドとしては、配列番号5により表されるオリゴヌクレオチドを例示できるが、これらに限定されるわけではない。

【0022】検出の対象とするDNAの調製、プライマーの作製及びそれを用いたPCR、プローブの調製及びそれを用いたハイブリダイゼーションは、常法に従って行うことができ、特別な方法を用いる必要はない。

【0023】なお、PCRに使用するプライマーは、両方が特異的にアニールするものである必要は必ずしもなく、一方は非特異的にアニールするものであってもよい。このような非特異的にアニールするプライマーの一例としては、配列番号1記載の塩基配列により表されるプライマーを挙げることができる。

[0024]

【実施例】〔実施例1〕配列番号39及び配列番号40記載の塩基配列により表されるオリゴヌクレオチドを利用して、臨床患者より分離された抗酸菌株8菌株(KPM 2201 T、KPM 2202、KPM 2203、KPM 2013、KPM 2014、KPM 198 8-5、KPM 2209、KPM 2212)のgyrB遺伝子配列を決定し2た。得られたgyrB配列と遅発育性マイコバクテリア同定用gyrB配列セット(配列番号7-38)を利用して分子系統学的解析により系統関係を推定した。分子系統学的解析は、分子系統学的解析用汎用プログラムClustal W(Thompson,J. D., D. G. Higgins,and T. J. Gibson. 199 4. Clustal W:improving thesensitivity of progressive multiple sequence alignment through sequencewei*

* ghting, positions-specific gap penalties and weigh t matrix choice. Nucleic Acids Res. 22: 4673-468 0.)あるいはPhylip (Felsenstein, J. 1993, PHYLIP(Ph ylogeny Inference Package) version 3.5c. Distribute d by the author, Department of Genetics, Universit y of Washington, Seattle, U.S.A.) 等を用い、両プロ グラムの使用説明書に従い、以下のように行った。配列 番号39及び配列番号40記載の塩基配列により表されるオ リゴヌクレオチドを使用して得られたgyrB配列と配列番 10 号7-38の遅発育性マイコバクテリア同定用gyrB配列セッ トをClustal Wプログラムにより多重整列ファイルを作 成する。多重整列させる際に用いるパラメーターの一例 は"Gap Open Penalty: 15.00;Gap Extension Penalty: 6.66; DNA weight matrix: IUB; DNA transition weigh t:0.5"である。得られた多重整列はアミノ酸配列から得 られる多重整列ファイルと比較し、問題のある箇所は訂 正する。次に、多重整列ファイルをもとに各配列間の遺 伝学的距離を算出する。算出には、Phylipのdnadistプ ログラムを使用する。算出はKimura 2-parameter model に従って行う。 得られた遺伝学的距離から近隣結合法 により系統樹を作成する。系統樹の分岐の確からしさは bootstrap確率を計算すること等により検定する。-方、上記8菌株を16S rRNA遺伝子を利用した手法及び生 化学的手法によっても同定した。以上の結果を表2に示 す。

【0025】 【表2】

株名	生化学的試験	16S rRNA 遺伝子	DNA 相同性試験
KPM 2201T	M. gordonae	M. gordonae	M. gordonae
KPM 2202	M. gastori	M. gordonae	M. gordonae
KPM 2203	M. gastori	M. gordonae	M. gordonae
KPM 2013	M. scrofulcerum	M. gordonae	新種
KPM 2014	M. scrofulcerum	M. gordonae	新種
KPM 1988-5	M. scrofulcerum	M. gordonae	新種
KPM 2209	M. scrofulcerum	M. gordonae	新種
KPM 2212	データーなし	M. gordonae	新種

【0026】表が示すように、上記8菌株のうち、KPM 2201T、KPM 2202、KPM 2203の3菌株は、マイコバクテリウム・ゴルドナエに属するものと同定されたが、残りのKPM 2013、KPM 2014、KPM 1988-5、KPM 2209、KPM 22 40 12の5菌株は、マイコバクテリウム・ゴルドナエと近縁ではあるが別種(新種)であることが示唆された(図3)。この結果を、検証するためにDNA-DNA交雑試験(Ezaki, T., Hashimoto, Y., Takeuchi, T., Yamamoto, H., Shu-Lin Liu, Matsui, K., & Yabuuchi, E(1988). J. Clin. Microbiol. 26, 1708-1713. Ezaki, T., Hashimoto, Y., Takeuchi & Yabuuchi, E(1989). Int. J. Syst. Bacteriol. 39, 224-229)を行ったところ新種であることが支持された。この結果は、遅発育性マイコバクテリア同定用gyrB配列セットが既知の株のみならず新50

種の株に対しても信頼性の高い結果を与えることを示している。

【0027】〔実施例2〕マイコバクテリウム・カンサシイとマイコバクテリウム・ガストリのgyrB遺伝子の塩基配列を比較し(図1)、マイコバクテリウム・カンサシイのgyrB遺伝子に特異的にアニールするプライマー(配列番号3)とマイコバクテリウム・ガストリに特異的にアニールするプライマー(配列番号5)を作製した。また、両菌種のgyrB遺伝子にアニールするブライマー(配列番号1)も作製した。

【0028】 これらのプライマーを用いて、臨床患者より単離された株 KPM 1004, KPM 1007, KPM KY256, KPM KY761, KPM KY768, KPM 1988-1, KPM 3502, KPM 3503の 菌体破砕液をついて PCRを行った。 PCR法による増幅条件

は次のとおりである。

[0029]

95 °C 10分 1サイクル

95 ℃ 1分、68 ℃ 1分30秒 30サイクル

72 °C 10分 1サイクル

プライマー濃度 各1 μΜ

dNTP

各 100 µM

Ampli Taq COLD™及び添付のPCR buffer Iを使用(米国 Perkin Elmer社)

【0030】増幅されたDNA 断片を電気泳動法により解 10 【0031】 析したところ、KPM 1004, KPM 1007, KPM KY256, KPM K*

* Y761, KPM KY768, KPM 1988-1は配列番号 l と3に示した 組み合わせでのみ増幅断片が観察されたので(表3)、 これらの株は マイコバクテリウム・カンサシイと同定 された。また、 KPM 3502, KPM 3503は配列番号1と5に 示した組み合わせでのみ増幅断片が観察されたので (表 3)、これらの株はマイコバクテリウム・ガストリ と 同定された。判定に用いた電気泳動像は図2に示すとお りである。これらの同定結果はDNA-DNA ハイブリダイゼ ーション法による同定結果と一致した。

【表3】

	<u>M. kansasii</u>	M. gastri
配列番号1	增幅可。	增幅不可。
配列番号3		建棚小山。
配列番号1	增幅不可。	增幅可。
配列番号5		THE CIO

[0032]

【発明の効果】本発明は、これまで同定することが困難 であった遅発育性マイコバクテリアの分類同定を正確に 20 となる。 行うことを実現するものである。また非結核性抗酸菌の うち16S rRNA 遺伝子配列に基づく同定法では鑑別が困 ※

※難であった菌種、例えばマイコバクテリウム・カンサシ イとマイコバクテリウム・ガストリイの迅速同定が可能

[0033]

【配列表】

SEQUENCE LISTING

<110> MARINE BIOTECHNOLOGY INSTITUTE CO., LTD

△20> DNA CYTRESU IDENSHTCHU NI SONZATSURU TOKUCHYOUTEKINA ENKIHATRETSU WO MOCHIITA CHIHATSUIKUSEI MAIKOBAKUTERIA NO DOUTEIHOU OYOBI TOKUITEKI K

Enshutsuhou - -

<130> P99-0373

<160> 40

<170⊳ PatentIn Ver. 2.0

<210> 1

<211> 20

<212> DNA

213> Artificial Sequence

<400> 1

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<210> 2

<211> 6

<212> PRT

<213> Artificial Sequence

<400> 2

Gly Val Ser Val Val Asn

<210> 3

<211> 20

<212> DNA

213> Artificial Sequence

<400> 3

gac ctt gtg cgg ggc ggc gg

<210> 4

<211> 6

192

```
<212> PRT
```

<213> Artificial Sequence

<400> 4

Ala Ala Pro His Lys Val

<210> 5

<211> 20

<212> DNA

<213> Artificial Sequence

<400> 5

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<210> 6

<211> 6

<21.2> PRT

<213> Artificial Sequence

<400> 6

Thr Ala Pro His Lys Val

<210> 7

<211> 1263

<212> DNA

<213> Mycobacterium simiae

<220>

<221> CDS

<222> (1)..(1263)

<400> 7

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5 10 1

gtg tcg gtg gtc aac gcc ctg tcc acc cgc ctg gaa gtc aac gtc aag 96 Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asn Val Lys

cgt gac ggc tat gag tgg ttc cag tac tac gac cgg gcg gtg ccc ggc 144 Arg Asp Gly Tyr Glu Trp Phe Gln Tyr Tyr Asp Arg Ala Val Pro Gly

Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg

ttc tgg gcc gat cct gag atc ttc gaa acc acc cag tac gac ttc gag 240 Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu

65 70 75 80 acg gtg gcg cgc cgg ttg cag gaa atg gcg ttc ctc aac aag ggc ctg 288

Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu 85 90 95

acc atc aac ctc acc gac gaa cgt gtc gag cag gac gag gtg gtc gat 336 Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp

100 105 110

gag gtg gtt agc gac acc gcc gag gcg ccg aag tca gcc gag gag cag 384 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Gln

115 120 125

gcg gcc gaa tcg gcc aag ccg cac aag gtc aag cac cgc acg ttc cac 43 Ala Ala Glu Ser Ala Lys Pro His Lys Val Lys His Arg Thr Phe His

130			14
	135	140	
tac ccg ggt ggg ttg	gtg gat ttc gtc	aag cac atc aat	cgc acc aaa 480
Tyr Pro Gly Gly Lec	ı Val Asp Phe Val	Lys His Ile Asn	Ara Thr Lvs
145	150	155	
aac ccq atc cag cag	age gte ate gae	ttc mac mor and	160
Asn Pro Ile Gin Gin	Ser Val Ilo Ace	The Asia GR.	gga acc ggg 528
165	oc. var Tie Asp		Gly Thr Gly
		170	175
cac gaa gtc gag atc	gcg atg cag tgg	aac ggt ggt tat	tog gag tog 576
His Glu Val Glu Ile	Ala Met Gln Trp	Asn Gly Gly Tyr !	Ser Glu Ser
180	185	1	190
gtg cac acc ttc gcc	aac acc atc aac	acc cat gag one o	100 acc cae
Val His Thr Phe Ala	Asn Thr Ile Asn	The His Clu Clu C	igc acc cac 624
195	200	_	ily inr His
gag gag ggc ttc cgc		205	
gag gag ggc ttc cgc	Com Ala Lug acc	teg gtg gtg aac a -	ag tac gcc 672
Glu Glu Gly Phe Arg	Ser Ala Leu Ihr	Ser Val Val Asn L	ys Tyr Ala
210	215	220	
aaa gac aag aag ctg	ctc aag gac aag g	pat ccc aac ctc a	cc ggc qac 720
Lys Asp Lys Lys Leu	Leu Lys Asp Lys /	Sp Pro Asn Leu 11	or Gly Asn
225	230	235	
gac atc cga gaa ggg	ctq qcc qca ata a		240
Asp Ile Arg Glu Gly I	eu Ala Ala Val T	le cee yey aay yi	c gcc gag 768
245			ıl Ala Glu
		50	255
ccg cag ttc gag ggc (ay act aag acg a	aa ctc ggc aac ac	c gag gtc 816
Pro Gln Phe Glu Gly (in the Lys The L	ys Leu Gly Asn Th	r Glu Val
260	265	27	٠ .
aag tog ttt gtc cag a	aa gtc tgt aac ga	aa caa ctc act ca	c tgg ttc 864
Lys Ser Phe Val Gin L	ys Val Cys Asn G	lu-Gin-Leu Thr Hi	S Tro Phē
275	280	285	- The
gag gcg aac ccg tcg g	aa gct aaa acc ot	t ata aac aan ac	7 att to
Glu Ala Asn Pro Ser G	lu Ala I vs Thr Va	of Val Ace the Ar	g gtt tcg 912
290	295		a vai Ser
tca acc can acc cac at		300	
tcg gcc cag gcc cgc at	r geg geg egt aa	g gog ogg gag tto	gtg cgg 960
Ser Ala Gln Ala Arg II	e Ala Ala Arg Ly	s Ala Arg Glu Leu	Val Arg
303 33	.0	315	330
cgt aag agt gct acg ga	t ttg ggt ggg tt	q ccg ggc aag ttg	gct gat 1008
Arg Lys Ser Ala Thr As	p Leu Gly Gly Le	Pro Civ Lvs Leu	Ala Asn
325	330		335
tgc cgc tcg acg gat cc	g cgg aag tot gad	l Cto tat oto oto	555
Cys Arg Ser Thr Asp Pro	O Aro Ivs Ser Clu	lou Tum Vez vez	gaa ggt 1056
340			Glu Gly
	345	350	
gat too gog ggt ggg to	i gcg aaa agt ggg	cgt gat tcg atg	ttc cag 1104
Asp Ser Ala Gly Gly Ser		Arg Asp Ser Met	Phe Gin
355	360	365	
ocg atc ttg ccg ctg cgc	ggc aag atc atc	aac gtc gaa aag	gcc cgc 1152
Ala Ile Leu Pro Leu Arg	Gly Lys Ile Ile	Asn Val Glu Lvs	Ala Aro
370	375	380	ru y
atc gat cgg gtg ctg aaa	aac acc gaa gtr	CAO OCC atc ata	350
Ile Asp Arg Val Leu Lys	Asn Thr Glu Val	Clo Ala Tia Ti	acc gcg 1200
385 390	III VIU VAI		
ctg ggc acc ggc atc cac	Gac gaa tto a	395	400
and and call	yar yaa LLC gac	dic acc aga cto d	of tac 1240

	1	L5		•											1	.6
Leu	Gly	Thr	· Gly	′ Ile	His	Asp	Glu	Phe	Asp	Πe	• Thr	Lys	Leu	ı Arç	Tyr	
				405					410)				415	;	
cac	aaq	ato	gto	ttg	1											1263
				Leu												
	_,-		420													
∠ 21	.0> 8															
	1> 1															
	2> D															
<21	3> M	ycob	acte	rium	bov	is										
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<22	1> C	DS														
<22	2> (1)	(125	7)												
<40	0> 8															
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Ser	Asp	Ala	Tyr	Ala	Ile	Ser	GΊγ	Gly	Leu	His	Gly	۷a۱	Gly	Val	Ser	
1				5					10					15		
ata	att	aac	aca	cta	tcc	acc	caa	ctc	gaa	atc	aaa	atc	aad	cgc	aac	96
														Arg		50
va i	vai	7311	20		<i>3</i> E1	1111	Aig		Giu	vai	Ulu	Tie			ASP	
	+							25					30			
														ggc		144
GIY	ıyr		Irp	Ser	Gin	Val		Glu	Lys	Ser	Glu	Pro	Leu	Gly	Leu	
		35					40					45				
aag	caa	ggg	gcg	ccg	acc	aaq	aag	acg	ggg	tca	acg	gta	cgg	ttc	tgg	192
Lys	Gln	Gly	Ala	Pro	Thr	Lys	Lys	Thr	Gly	Ser	Thr	۷a٦	Arg	Phe	Trp	
	50					55					60					٠
gcc	gac	ссс	qct	gtt	ttc	gaa	acc	acg	gaa	tac	gac	ttc	gaa	acc	gtc	240
Ala	Asp	Pro	Ala	Val	Phe	Glu	Thr	Thr	Glu	Tyr	Asp	Phe	Glu	Thr	Val	
65					70					75					80	
qcc	cqc	cga	ctq	caa	gag	atq	aca	ttc	ctc	aac	aaa	aaa	cta	acc	atc	288
_														Thr		
				85					90		_,_	. .,		95		
aac	cta	acc	aac		200	ata	200	C22		a aa	atc	atc	026	gaa	ata	226
																336
ASII	LCu	1111		Giu	Aig	vai	HIII.		ASp	Giù	Vai	Vai		Glu	vai	
			100					105					110			
														gca		384
Val	Ser	Asp	Val	Ala	Glu	Ala	Pro	Lys	Ser	Ala	Ser	Glu	Arg	Ala	Ala	
		115					120					125				
gaa	tcc	act	gca	ccg	cac	aaa	gtt	aag	agc	cgc	acc	ttt	cac	tat	ccg	432
Glu	Ser	Thr	Ala	Pro	His	Lys	Val	Lys	Ser	Arg	Thr	Phe	His	Tyr	Pro	
	130					135					140					
ggt	ggc	ctg	qtq	gac	ttc	gtg	aaa	cac	atc	aac	cgc	acc	aaq	aac	qcq	480
														Asn		
145					150		·			155	• •		_,_		160	
	cat	aar	aac	atc		gac	ttt	tcc	aac		αœ	acc	000	cac		528
																220
116	3	261	261		٧dI	Λομ	THE			LYS	UIY	our.	σιγ	His	oiu	
		_4-		165					170					175		
														gtg		576
Val	Glu			Met	Gln	Trp	Asn	Ala	Gly	Tyr	Ser	Glu	Ser	Val	His	
			180					185					190			
acc	ttc	gcc	aac	acc	atc	aac	acc	cac	gag	ggc	ggc	acc	cac	qaa	gag	624

Q

The Phe Ala Asn The Ile Asn The His Clu Cly Cly The His Cl	
Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu	J
203	
ggc ttc cgc agc gcg ctg acg tcg gtg gtg aac aag tac gcc aag gac	672
Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp)
210 215 220	
cgc aag cta ctg aag gac aag gac ccc aac ctc acc ggt gac gat atc	720
Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile	
225 230 235 240	
cgg gaa ggc ctg gcc gct gtg atc tcg gtg aag gtc agc gaa ccg cag	
Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln	768
245	
250 255	
ttc gag ggc cag acc aag acc aag ttg ggc aac acc gag gtc aaa tcg	816
Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser	
260 265 270	
ttt gtg cag aag gtc tgt aat gaa cag ctg acc cac tgg ttt gaa gcc	864
Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala	
275 280 285	
aac ccc acc gac tog aaa gto gtt gtg aac aag got gtg too tog gog	912
Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala	J12
290 295 300	
caa gcc cgt atc gcg gca cgt aag gca cga gag ttg gtg cgg cgt aag	
Gin Ala Arg Tie Ala Ala Arg Luc Ala Arg Gu Luc Ala Arg	960
Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Giu Leu Val Arg Arg Lys	
310 313 320	
age gee ace gae ate ggt gga ttg eee gge aag etg gee gat tge egt	1008
Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg	•
325 330 335	
tcc acq gat ccg cgc aag tcc gaa ctg tat gtc gta gaa ggt gac tcg	1056
Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser	
340 345 350	
get age the year and age ggt ege gat teg atg the can men ata	1104
gcc ggc ggt tct gca aaa agc ggt cgc gat tcg atg ttc cag gcg ata Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Glo Ala Tlo	1104
Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile	1104
Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 355 360 365	
Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 355 360 365 ctt ccg ctg cgc ggc aag atc atc aat gtg gag aaa gcg cgc atc gac	1104
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Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 355 360 365 ctt ccg ctg cgc ggc aag atc atc aat gtg gag aaa gcg cgc atc gac Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 370 375 380	
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Arg Asp Gly Tyr Met Trp Ser Gln Phe Tyr Asp His Ala Glu Pro Gly	•
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acc ctc aaa cag ggc gag gcc acc aag acg acg gga acc acc	כ
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Thr	Leu 50		G]n	Gly	/ GI	Ala 55		Lys	Thr	Thr			Thr	· Ile	e Arg	
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Thr	Ile	Asn			Asp	Glu	Arg			Glu	Glu	Glu	۷a۱	Val	Asp	
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ASP	vai	va i		ASP	inr	Ala			Pro	Lys	Ser			Glu	Lys	
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Cys .	Arg	ser	340	ASp	Pro	Arg	Lys		Giu	Leu	ıyr			Glu	Gly	
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							aag Lys									1104
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								_	-				-,-			

(16)29 30 370 375 atc gac cgg gtg ttg aag aac acc gag gtg cag gcc atc atc acc gcc Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala 390 395 ctg ggc acc ggc atc cac gac gag ttc gac atc acc aag ctg cgc tat 1248 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr 410 cac aag atc gtg ctg 1263 His Lys Ile Val Leu 420 <210> 12 <211> 1263 <212> DNA <213> Mycobacterium avium <220> <221> CDS <222> (1)..(1263) <400> 12 ggc gag aac agc ggc tac aac gtc agc ggc ggt ctg cac ggc gtc ggc Gly Glu Asn Ser Gly Tyr Asn Val Ser Gly Gly Leu His Gly Val Gly 10 gtc tcg gtg gtc aac gcg ctg tcc act cgg ctc gag gtc aac atc gcc Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asn Ile Ala 25 cgc gac ggc tac gag tgg tcg cag tac tac gac cac gcc gtg ccc ggc Arg Asp Gly Tyr Glu Trp Ser Gln Tyr Tyr Asp His Ala Val Pro Gly Thr Leu Lys Gln Gly Glu Ala Thr Lys Arg Thr Gly Thr Thr Ile Arg 55 ttc tgg gcc gac ccc gac atc ttc gag acc acc gag tac gac ttc gaa 240 Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu 70 75 acg gtg gcc cgg cgg ctg cag gaa atg gcg ttc ctc aac aag ggc ctg Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu 85 acc atc aac ctc acc gac gag cgg gtg acc aac gaa gag gtc gtc gac Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Asn Glu Glu Val Val Asp 100 105 gag gtg gtc agc gac acc gcc gac gca ccc aag tcg gcg cag gag aag Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Gln Glu Lys 115

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Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
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	•	
⋖	•	

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&b	ser		Gly	GIY	ser	Ala		ser	GIY	Arg	Asp		met	Phe	GIn	
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			ccg													1152
NI d		Leu	Pro	Leu			LV5	116	тıе	ASN		Glu	Lys	Ala	Arg	
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			gtg													1200
	ASp	Arg	Val			ASTI	INC	Giu	vaı		АІа	Tie	Пe	ihr		
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			999													1248
.eu	uly	mr	Gly :		H1S	ASP	ulu			пе	ınr	LYS			lyr	
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Thr Leu Lys Gln Gly Glu Thr Thr Arg Lys Thr Gly Thr Thr Ile Arg
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110

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Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His

195

200

205

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FIU	Gill	PHE			Gin	Inr	Lys		Lys	Leu	GIY	ASN			vaı	
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Lys	ser			GIN	Lys	vai			Glu	GIN	Leu		HIS	1 rp	Phe	
93.9	966	275		+			280					285				
									gtt							912
Giu		ASN	Pro	Ser	Glu		Lys	ınr	Val	vaı		Lys	АІа	Val	Ser	
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Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu	
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Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu	
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Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg	1000
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Ile	His	s Se	r Se	r Ile	e Vai	l Asp	Phe	Ser	- GTy	/ Lvs	Gly	Thi	· Glv	/ His	s Glu	. ,,
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۷a٦	Glu	H	e Ala	a Met	: Glr	Τη	Asn	Ala	Gly	/ Tyr	Ser	Glu	, Ser	· Val	His	370
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Thr	Phe	: Ala	a Asr	Thr	· Ile	Asn	Thr	His	Glu	Gly	Gly	Thr	His	Glu	பெ	02.4
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IJу	Phe	Arc) Ser	· Ala	Leu	Thr	Ser	Val	Val	Asn	Lys	Tyr	Ala	Lvs	Asp	
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Phe	Glu	Gly	Gln	Thr	Lys	Thr	Lys	Leu	Gly	Asn	Thr (Glu	Val	Lys	Ser	
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he ۱	/al	Gln	Lys	۷a٦	Cys	Asn	Glu	Gìn	Leu	Thr I	His 1	Ггр	Phe	Glu .	Ala	
		275					280					285				
			_		- =		. 9					n .=				·
ac c	·cc	act	ast	act												

aac ccc gct gat gct aaa acc gtt gtc aac aag gca gtt tca tcg gcg Asn Pro Ala Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser Ser Ala 290 cag gcc agg att gcg gcc cgc aag gcg cgc gag ttg gtg cgc cgc aag Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys 310 315 age gea ace gat etg gge gga eta eeg gge aag ttg gee gae tge ege 1008 Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg tcg acc gac ccc cgt aag tcc gaa tta tat gtg gtg gag ggt gat tca 1056 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser gcc ggc ggc tcg gcg aag agc ggc cgc gac tcg atg ttt caa gcg atc Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 360 ttg ccg ttg cgc ggc aag atc atc aac gtc gag aag gcc cgc atc gac 1152 Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 370 375 cgg gtg ctg aag aac acc gaa gtc cag gcg atc atc acc gcg ttg ggc Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly 385 395 acc ggt att cac gac gaa ttc gac atc gcg aga ctg cgt tac cac aag

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 atc gtg ctg
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Gly Glu Asn Ser Gly Tyr Asn Val Ser Gly Gly Leu His Gly Val Gly
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gtg tcg gtg gtc aac gcg ctg tcc acc cga ctg gaa gtc gac atc aag
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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys
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                                 25
cgc gac gga tac gag tgg tcg cag ttc tac gac cgc gcc cag ccg ggc
Arg Asp Gly Tyr Glu Trp Ser Gln Phe Tyr Asp Arg Ala Gln Pro Gly
                             40
192
Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
                         55
ttc tog gcc gac tcg gac atc ttt gag acc acc gaa tac gac ttc gag
                                                                 240 1
Phe Trp Ala Asp Ser Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
acg gtg gcg cgg cgc ctg cag gag atg gcg ttc ctc aac aag ggc ctg
                                                                288
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                85
                                    90
acc atc aac ctc acc gac gag cgg gtc acc ccg gac gag gtc gtc gac
                                                                336
Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Pro Asp Glu Val Val Asp
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gac gtc gtc agt gat acc gcc gaa gca cca aag tcc gcc cag gag aag
Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Gln Glu Lys
        115
                           120
gcc gcc gaa tcg acc gcg ccg cac aag gtc aag agc cgc acc ttc cac
Ala Ala Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His
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tat ccc ggc ggt ttg gtc gat ttc gtc aag cac atc aac cgc acc aag
Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
145
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agt ccg att cag cag agc atc gtc gac ttc gag ggc aag ggc tcc ggc
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Ser Pro Ile Gln Gln Ser Ile Val Asp Phe Glu Gly Lys Gly Ser Gly
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                                   170
cac gaa gtc gaa atc gcg atg cag tgg aac ggc ggc tac tcg gag tcg
His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
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gtg cac acc ttc gcc aac acc atc aac acc cat gag ggt gga acg cac
Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
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  aaa gac aag aag ctg ctc aag gac aag gac ccc aac ctc acc ggt gac
  Lys Asp Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
  225
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  gac atc cgc gag ggg ttg gcc gcg gtc atc tcg gtg cgg gtg gca gag
  Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Arg Val Ala Glu
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                                      250
  ccg cag ttc gag ggt cag acg aag acc aag ctg ggc aac acc gag gtc
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  Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
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 aag tcg ttt gtc cag aag gtt tgt aac gag cag ctc acc cac tgg ttc
 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
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 gag gcc aat cct tcg gaa gcc aaa acc att gtg aac aag gcg gta tcc
 Glu Ala Asn Pro Ser Glu Ala Lys Thr Ile Val Asn Lys Ala Val Ser
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 tog gog cag goa ogt etc god gog ogc aag gog oga gag tig gig ogt
 Ser Ala Gln Ala Arg Leu Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
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 cgc aag agc gca acc gat ctc ggt ggg ctg ccc ggc aag ttg gcc gac
                                                                   1008
 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                 325
 tgc cgc tcg aca gat ccg cgt aag tcg gaa ctg tat gtg gtg gag ggt
 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr-Val-Val-Glu-Gly
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                                 345
gac tcg gcc ggc ggc tcg gca aag agt ggc cgc gat tcg atg ttc cag
Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
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                             360
gcg atc ctg ccg ctg cgc ggc aag atc atc aat gtc gaa aag gca cgc
Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
atc gac cga gtc ctg aaa aac act gaa gtc cag gcg atc atc acc gcg
                                                                  1200
Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
                    390
ttg ggt acc ggt att cac gac gaa ttc gac ctc tcg aag ctg cgc tat
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Leu Gly Thr Gly Ile His Asp Glu Phe Asp Leu Ser Lys Leu Arg Tyr
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                                                        415
cac aag atc gtc ttg
                                                                  1263
His Lys Ile Val Leu
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Ser Asp A	la Tyr A	la Ile Se	r Gly	Gly Leu	His G	y Val	Gly Val	Ser	
1		5		10			15	i	
gtg gtt aa	ac gcg c	ta tcc ac	c cgg	ctc gaa	gtc ga	g atc	aag cgc	gac 96	5
Val Val As									
	20		.,	25 .			30	, . – ,-	
ggg tat ga	a taa t	ct caa at	t tat		tca aa	a ccc		ctc 14	14
Gly Tyr G									
	35	c. c •c	40	010 273	JC1 01	45	ccu diy	Leu	
		כת פכר פפ		2C0 000	tca ac		coa tto	tag 10	רי
aag caa go									12
Lys Gln Gl	y Ala P			inr Giy			Arg Phe	: irp	
50			5		. 6				_
gcc gac co								• •	0
Ala Asp Pr	o Ala V		u Thr	Thr Glu	Tyr Ası	Phe	Glu Thr	Val	
65		70			75			80	
dec ede ed									8
Ala Arg Ar	g Leu G	in Glu Me	t Ala I	Phe Leu	Asn Ly	s Gly	Leu Thr	Ile	
	8	35		90			95	i	
aac ctg ac	c gac ga	ag agg gt	g acc o	caa gac	gag gto	gtc	gac gaa	gtg 33	6
Asn Leu Th	r Asp G	lu Arg Va	Thr (Gln Asp	Glu Va	ا Val	Asp Glu	Val	
	100		1	105			110		
gtc agc ga	c gtc go	c gag go	g ccg a	aag tog	gca agi	gaa (cgc gca	gcc 384	4
Val Ser As	p Val Al	la Glu Al	a Pro l	Lys Ser	Ala Sei	r Glu /	Arg Ala	Ala	
11			120			125			
gaa tcc ac	t gca co	g cac aa	a gtt a	aag agc	cqc acc	ttt (cac tat	ccq 432	2
Glu Ser Th								• • •	
130		13		•	140				
ggt ggc ct	a ata aa			ac atc			aan aac	qcq 480	n
Gly Gly Le									•
145	,	150	,5 .		155	,	Ly3 AGII	160	
att cat ag	c age at		- +++ +			. 200	777 626		0
									3
Ile His Se) rile 3		Lys Giy	mr C		Giu	
	16			170			175		
gtg gag at					-				5
Val Glu Il		t Gin In			Tyr Ser			His	
	180			185			L90		
acc ttc gc									4
Thr Phe Al	a Asn Th	r Ile Asr	Thr H	lis Glu	CTV CTV	Thr H	tis Glu	Glu	
19	5		200			205			
ggc ttc cg	c agc gc	g ctg aco	tcg g	to ata	aac aag	tac c	acc aag	gac 672	2
Gly Phe Ar	g Ser Al	a Leu Thi	· Ser V	al Val .	Asn Lys	Tyr A	la Lys	Asp	
210		215	5		220)			
cgc aag ct	a ctg aa	g gac aag	gac c	cc aac	ctc acc	ggt c	ac gat	atc 720)
Arg Lys Le									
225		230			235	•		240	
cgg gaa gg	cta ac	c gct ata	atc t			agc c	aa cco		3
Arg Glu Gl							•	• •	
	24			250	_,	`	255		
		-							

ttc gag ggc cag acc aag acc aag ttg ggc aac acc gag gtc aaa tcg ~ 816

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54
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Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser
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  Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala
          275
  aac ccc acc gac tcg aaa gtc gtt gtg aac aag gct gtg tcc tcg gcg
  Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala
                         295
 caa gcc cgt atc gcg gca cgt aag gca cga gag ttg gtg cgg cgt aag
 Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys
                     310
                                         315
 age gee ace gae ate ggt gga ttg eee gge aag etg gee gat tge egt
                                                                 1008
 Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg
                                     330
 tcc acg gat ccg cgc aag tcc gaa ctg tat gtc gta gaa ggt gac tcg
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 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser
             340
                                345
 gcc ggc ggt tot gca aaa agc ggt ogc gat tog atg tto cag gcg ata
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 Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile
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                            360
 ctt ccg ctg cgc ggc aag atc atc aat gtg gag aaa gcg cgc atc gac
 Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp
     370
                        375
 cgg gtg cta aag aac acc gaa gtt cag gcg atc atc acg gcg ctg ggc
                                                                 1200
 Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly
 385
                    390
                                        395
 acc ggg atc cac gac gag ttc gat atc ggc aag ctg cgc tac cac aag, 1248
 Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys
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                                    410
 atc gtg ctg
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Ile Val Leu
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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys
cgc gac ggg cac gag tgg tcc cag tat tac gag cgc gcc gtt cct ggc
                                                                144
Arg Asp Gly His Glu Trp Ser Gln Tyr Tyr Glu Arg Ala Val Pro Gly
Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
```

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	50)				55	;				60)				
tto	tgg	gcg	gac	ccg	gac	atc	tto	gaç	acc	acc	cag	tac	gad	tto	qaq	24
Phe	Trp	Ala	Asp	Pro	Asp	Ile	Phe	์ ได้ใน	Thr	Thr	Gln	Tyr	· Asp	Phe	e Glu	
65	i				70)				75	i				80	
aco	gtg	gcg	cgc	cgg	ctc	caa	gag	atg	gcg	ttc	ctg	aac	aaç	qqq	ttg	28
Thr	· Val	Ala	Arg	Arg	Leu	Gln	Glu	Met	Ala	Phe	Leu	Asn	Lys	Gly	Leu	
				85					90)				95	;	
acc	atc	aac	ttg	acc	gac	gag	cgg	gtg	gac	cag	gac	gag	gto	gto	gat	33
Thr	Ile	Asn	Leu	Thr	Asp	Glu	Arg	Val	Asp	Gln	Asp	G٦u	Val	۷a۱	Asp	
			100					105					110)		
gaa	gtc	gtc	agc	gac	acc	gcc	gat	gcg	ccc	aaq	tcc	gcc	gaa	gaç	aag	38
G٦u	۷a۱	Val	Ser	Asp	Thr	A٦a	Asp	Ala	Pro	Lys	Ser	Ala	Glu	Glu	Lys	
		115					120					125				
gcg	gcc	gaa	tcc	aaa	gcg	ccg	cac	aag	gtt	aag	cac	cgc	acc	ttc	·cac	43
Аlа	Ala	Glu	Ser	Lys	Ala	Pro	His	Lys	۷a۱	Lys	His	Arg	Thr	Phe	His	
	130					135					140					
tac	ccc	ggc	ggc	ttg	gtc	gac	ttc	gtc	aag	cac	atc	aac	cgg	acc	aag	48
Tyr	Pro	Gly	G٦y	Leu	Val	Asp	Phe	Val	Lys	His	Ile	Asn	Arg	Thr	Lys	
145					150					155					160	
agc	ccg	atc	caa	cag	agc	gtc	atc	gac	ttc	gag	ggc	aaa	ggc	acc	qqc	52
Ser	Pro	Ile	Gln	Gln	Ser	۷a۱	Ile	Asp	Phe	Glu	Gly	Lys	Gly	Thr	Gly	
				165					170					175		
cac	gag	gtc	gag	atc	gcg	atg	cag	tgg	aac	ggt	ggc	tac	tcg	gag	tcg	57
His	G٦u	۷a٦	G٦u	Пe	Ala	Met	Gln	Trp	Asn	IJу	G٦y	Tyr	Ser	Glu	Ser	
			180					185					190			
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۷a٦	His	Thr	Phe	Ala	Asn	Thr	Ile	Asn	Thr	His	Glu	Gly	Gly	Thr	His	
		195					200					205				
gaa	gaa	ggg	ttc	cgc	agt	gcg	ctg	acg	tcg	gtg	gtq	aac	aaa	tac	gcc	67
Glu	Glu	Gly	Phe	Arg	Ser	Ala	Leu	Thr	Ser	Val	Val	Asn	Lys	Tyr	Ala	
	210					215					220					
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Lys	Asp	Lys	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	Gly	Asp	
225					230					235					240	
gac	atc	cgc	gag	gga	ctg	gcc	gcg	gtg	atc	tcg	gtc	aag	gtc	gcc	gaa	768
Asp	IJе	Arg	Glu	Gly	Leu	Ala	Ala	۷a٦	Ile	Ser	۷a۱	Lys	۷a٦	Ala	Glu	
				245					250					255		
ccc	cag	ttc	gag	ggc	cag	aca	aag	acc	aag	ctg	ggc	aac	acc	qaq	gtc	816
Pro	Gln	Phe	Glu	Gly	GIn	Thr	Lys	Thr	Lys	Leu	Gly	Asn	Thr	Glu	Val	
			260					265					270			
aag	tcg	ttc	gtg	cag	aag	gtg	tgc	aac	gaa	cag	ctc	acc	cac	tgg	ttc	864
Lys	Ser	Phe	Val	Gln	Lys	Vaì	Cys	Asn	Glu	Gln	Leu	Thr	His	Trp	Phe	
		275					280					285				
gag	gcc	aat	ccg	tcg	gaa	gcc	aaa	acc	gtt	gtc	aac	aag	gcg	gtt	tcg	912
	Ala															
	290					295				•	300					
tcc	gca	cag	gcc	cgg	atc	gcg	gcg	cgg	aag	qcc	cga	qaq	ttg	gtg	cgg	960
	Ala															

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gac tcg gca ggt ggc tcg gcc aag agc ggc cgt gac tcg atg ttc cag 1104 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln 355

gcc atc ctg ccg ctg cgc ggc aag atc atc aac gtc gag aag gcc cgc 1152 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 375

atc gac egg gtc etg aag aac acc gaa gtc eag geg atc atc acc geg Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala 385 390

ctg ggt acc ggt att cac gac gag ttc gac att tct aaa ctg cgt tac 1248 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Ser Lys Leu Arg Tyr 410

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cgc gac ggg cac gag tgg ttt cag tac tac gac cgc gcc gtg ccc gga Arg Asp Gly His Glu Trp Phe Gln Tyr Tyr Asp Arg Ala Val Pro Gly 40

acc ctc aag cag ggc gag gcc acc aag aag acc gga acc acg atc agg 192 Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg

ttc tgg gcg gac ccc gaa atc ttc gaa acc aca cag tac gac ttc gag 240 Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu

acc gtg gcg cgg cgg ctg cag gag atg gcc ttc ctc aac aag ggc ctc 288

Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu

acc atc aac ctc acc gac gaa cga gtg gag cag gac gag gtc gtc gac Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp

đ	п		

			100					105					110	}		
gag	gtc	gtc	agc	gac	acc	gcc	gag	gca	ccg	aag	tcc	gcc	gaa	gaq	aag	384
ପାu	Val	۷a٦	Ser	Asp	Thr	Ala	Glu	Ala	Pro	Lys	Ser	Аlа	Glu	Glu	Lys	
		115					120	1				125				
gcc	gcg	gaa	tcg	act	qcq	cca	cac	aag	gtc	aag	cac	cgc	acc	ttc	cac	432
Ala	Ala	Glu	Ser	Thr	Ala	Pro	His	Lys	Vaì	Lys	His	Arg	Thr	Phe	His	
	130					135					140					
tac	ccc	ggc	ggt	ctg	gtc	gac	ttc	gto	aag	cac	atc	aac	cgc	acc	aag	480
Tyr	Pro	Gly	Gly	Leu	۷a٦	Asp	Phe	۷a٦	Lys	His	Ile	Asn	Arg	Thr	Lys	
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Ser	Pro	Пe	Gìn	Gln	Ser	Val	Ile	Asp	Phe	Asp	Gly	Lys	ĢΊγ	Thr	Gly	
				165					170					175		
cac	gag	gtc	gag	atc	gcc	atg	cag	tgg	aac	ggc	ggc	tac	tcg	gag	·tcc	576
His	G٦u	۷a۱	Glu	Ile	Ala	Met	Gln	Trp	Asn	GΊγ	Gly	Tyr	Ser	Glu	Ser	
			180					185					190			
gtc	cac	acc	ttc	gcc	aac	acc	atc	aac	acg	cac	gag	ggc	ggc	acc	cac	624
Val	His	Thr	Phe	Ala	Asn	Thr	Ile	Asn	Thr	His	Glu	G٦y	G٦y	Thr	His	
		195					200					205				
gag	gag	ggc	ttc	cgc	agc	gcg	ctg	ácg	tcg	gtg	gtg	aac	aag	tac	gcc	672
Glu	Glu	Gly	Phe	Arg	Ser	Ala	Leu	Thr	Ser	۷a۱	۷a٦	Asn	Lys	Tyr	Ala	
	210					215					220					
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Lys	Asp	Lys	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	Gly	Asp	
225					230					235					240	
gac	atc	cgt	gag	ggc	ttg	gcc	gcg	gtc	atc	tcg	gtg	aag	gtc	gcc	gag	768
Asp	Ile	Arg	Glu	GΊγ	Leu	Ala	Ala	۷a٦	IJе	Ser	Val	Lys	Val	Ala	Glu	
				245					250					255		
cca	cag	ttc	gaa	ggc	cag	acc	aag	aca	aag	ctg	ggc	aac	acc	gag	gtg	816
Pro	Gln	Phe	Glu	Gly	GIn	Thr	Lys	Thr	Lys	Leu	Gly	Asn	Thr	Glu	Val	
			260					265					270			
aaq	tcg	ttc	qtq	cag	aag	gtg	tgc	aac	gag	cag	ctc	acc	cac	tgg	ttc	864
Lys	Ser	Phe	Val	G1n	Lys	Val	Cys	Asn	Glu	Gln	Leu	Thr	His	Trp	Phe	
		275					280					285				
gag	gcc	aac	cca	tcc	gag	gcg	aaa	acg	gtg	gtg	aac	aaa	gcg	ata	tcg	912
۵lu	Ala	Asn	Pro	Ser	Glu	Ala	Lys	Thr	Val	Val	Asn	Lys	Ala	Val	Ser	
	290					295					300					
tcg	gct	cag	gcg	cgc	att	qcc	gcc	cgc	aag	gcg	cgt	gaa	ctg	gtg	cgc	960
Ser	Ala	Gln	Ala	Arg	Пe	Ala	Ala	Arg	Lys	Ala	Arg	Glu	Leu	Val	Arg	
305					310					315					320	
cgc	aag	agc	gcc	acc	gac	ctc	ggc	ggt	ctg	ccc	ggg	aag	ctg	gcc	gac	1008
Arg	Lys	Ser	Ala	Thr	Asp	Leu	Gly	Gly	Leu	Pro	Gly	Lys	Leu	Ala	Asp	
				325					330					335		
tgc	cgc	tcc	acc	gac	ccg	cgg	aaa	tcg	gaa	ctg	tat	gtg	gtg	gag	ggc	1056
		Ser														
			340					345					350		•	
gat	tcg	gcc	ggc	ggc	tcg	gcc	aaq		ggq	cgc	gac	tca		ttc	caq	1104
		Ala														
		355	-	-			360		·	••	•	365		-		

1263

gcg atc ctg ccg ctg cgc ggc aag atc atc aat gtc gag aag gcc cgc 1152 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 370 375 380

atc gac cgg gtg ctg aag aac acc gaa gtt cag gcg atc atc acc gcg

1200

11e Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala

385

390

395

400

ctg ggt acc ggg att cac gac gag ttc gac atc acc aag ctg cgc tat 1248 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr

05 410 415

cac aag atc gtg ctg

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<220>

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<222> (1)..(1260)

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5 10 1

acc gac ggg tac gag tgg ttt cag cat tac gac cgc tct gtc ccc ggc 144
Thr Asp Gly Tyr Glu Trp Phe Gln His Tyr Asp Arg Ser Val Pro Gly
35 40 45

ttc tgg gcc gac ccg gac atc ttc gag acg acg gat tac gac ttc gag 24
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Asp Tyr Asp Phe Glu

65 70 75 80

acg gtc gca cgc cgg ctg cag gaa atg gcg ttc ctc aac aaa ggg ctg 288
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
85

acc atc aac ctg acc gac gag cgg gtg cga aac gaa gaa gtc gtc gac 336 Thr Ile Asn Leu Thr Asp Glu Arg Val Arg Asn Glu Glu Val Val Asp

9ag gtc gtc agc gac acc gcc gac gcg ccg aag tcg gcg cgc gaa gag 384

Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Arg Glu Glu
115 120 125

gcc gaa gaa cgg acc acg cag aaa gtc aag cac cgc acg ttc cat tac 432 Ala Glu Glu Arg Thr Thr Gln Lys Val Lys His Arg Thr Phe His Tyr 130 135 140

ccc ggc ggc ttg gtc gat ttc gtc aaa cac atc aac cgc aca aag aac 480

	•														U	4
Pro	Gly	Gly	/ Leu	Va1	Asp	Phe	e Val	Lys	His	: Ile	2 Asn	Arç	Thi	Lys	Asn	
145					150					155					160	
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Pro	Ile	His	Ser			· Val	Asp	Phe			' Lys	Gly	Pro		His	
a 20	ata			165					1.70					175		
															gtg Val	576
U.u	vai	Giu	180		MEL	. 011	iiip	185		Giy	1 91	Sei	190		VdI	
cac	acc	ttc			acc	ato	aac			oso:	aac	aac			gaa	624
															Glu	02.4
		195					200				·	205				
gaa	ggg	ttc	cgc	qcq	gca	ctg	acg	tcc	qtq	gtg	aac	aag	tac	gcc	aag	672
Glu	Gly	Phe	Arg	Ala	Ala	Leu	Thr	Ser	۷a٦	Va1	Asn	Lys	Tyr	Ala	Lys	
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	Arg	Lys	Leu	Leu			Lys	Asp	Pro			Thr	Gly	Asp	Asp	
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							gtc Val								ccg	768
116	Ai 9	Giu	Giy	245	Ala	Ala	vai	116	250		LyS	Vai	Ser.	255	Pro	
									2 30					233		
cag	ttc	gag	ggc	cag	acc	aaa	acc	aaa	ctc	qqc	aac	acc	qaa	atc	aaq	816
							Thr									
			260					265					270			
tcg	ttt	gtg	cag	aag	gtc	tgc	aac	gaa	cag	ctc	acc	cac	tgg	ttc	gag	864
Ser	Phe	Va1	Gln	Lys	۷a۱	Cys	Asn	Glu	Gln	Leu	Thr	His	Trp	Phe	Glu	
		275					280					285				
							acc									912
АІА	290	Pro	Ser	ASP	Ala		Thr	vai	Val	Asn		Ala	Val	Ser	Ser	
oca		occ	cac	att	occ	295	cgc	222	000	CO 2	300	++-	ata			060
							Arg									960
305	• • • • • • • • • • • • • • • • • • • •	,,,,	, w 94		310	, tiu	Λ' 9	Lys	Λια	315	Olu	Leu	vai	Aig	320	
	agc	gca	acc	gat		qqc	999	cta	cca		aaa	cta	act	gac		1008
							Gly									
				325					330					335	,	
cgc	tcq	acc	gat	cca	cgc	aag	tcc	gaa	ttg	tat	gtg	gtg	gag	ggt	gat	1056
Arg	Ser	Thr	Asp	Pro	Arg	Lys	Ser	G٦u	Leu	Tyr	۷a٦	Val	Glu	Gly	Asp	
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							agc									1104
Ser	Ala		Gly	Ser	Ala	Lys	Ser	Gly	Arg	Asp	Ser	Met	Phe	Gln	Ala	
		355					360					365				
							atc									1152
rie	370	PIO	Leu	Arg	GIY	375	Ile	Tie	ASn	vai		Lys	АІА	arg	Tie	
aac		ata	cta	aad	aac		gag	ata	can	oca	380 atc	atc	366	000	cta	1200
							Glu									1200
385				-,-	390			· • •		395				- v i u	400	
ggc	acc	ggg	att	cac		gag	ttc	gac	atc		aag	cta	cgc	tac		1248
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aag atc gtg ctg Lys Ile Val Leu

1260

240 .

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<210> 22

<211> 1263

<212> DNA

<213> Mycobacterium paratuberculosis

405

<220>

<221> CDS

<222> (1)..(1263)

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Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asn Ile Ala

cgc gac ggc tac gag tgg tcg cag tac tac gac cac gcc gtg ccc ggc
Arg Asp Gly Tyr Glu Trp Ser Gln Tyr Tyr Asp His Ala Val Pro Gly
35

ttc tgg gcc gac ccc gac atc ttc gag acc acc gag tac gac ttc gaa
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
65 -70 -75 -75 -75 -75 -75 -75

acg gtg gcc cgg cgg ctg cag gaa atg gcg ttc ctc aac aag ggc ctg 2
Thr Val Ala Arg Arg Leu Gîn Gîu Met Ala Phe Leu Asn Lys Gîy Leu

85 90 0F

acc atc aac ctc acc gac gag cgg gtg acc aac gaa gag gtc gtc gac 336
Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Asn Glu Glu Val Val Asp
100 105 110

gag gtg gtc agc gac acc gcc gac gca ccc aag tcg gcg cag gag aag
Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Gln Glu Lys
115
120
125

gcg gcg gaa tcg gct gcg ccg cat aag gtc aag cac cgc acc ttc cac 432 Ala Ala Glu Ser Ala Ala Pro His Lys Val Lys His Arg Thr Phe His

tac ccc ggc ggc ctg gtc gac ttc gtc aaa cac atc aat cgc acc aaa 480 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys

145 150 155 160 aac ccc atc cac cag agc atc atc gat ttc ggt ggg aag ggc ccc ggc 528

Asn Pro Ile His Gln Ser Ile Ile Asp Phe Gly Gly Lys Gly Pro Gly

165 170 175

cac gag gtc gag atc gcg atg cag tgg aac ggc ggc tac tcc gaa tcg 576 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 185 190

gtg cac acc ttc gcc aac acc atc aac acg cac gag ggc ggc acc cac 624 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His

67

<400> 23

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70	

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Val Ser Va	al Val Asn Ala Leu	Ser Thr Arg Lou	Glu Val Asn Val Lys
	20	25	
		23	30
Arg Asp Cl	v Tun Clu T N		
way rop u	y iyir didi impi me	Gin Tyr Tyr Asp	Arg Ala Val Pro Gly
3	15	40	45
ihr Leu Ly	's Gln Gly Glu Ala	Thr Lys Lys Thr	Gly Thr Thr Ile Arg
20	55		· 60
Phe Trp Al	a Asp Pro Glu Ile	Phe Glu Thr Thr	Gln Tyr Asp Phe Glu
65	70	75	
Thr Val Al	· -		
	85		Leu Asn Lys Gly Leu
The Ilo Ac		90	95
III TIE ASI	Leu inr Asp Glu ,	Arg Val Glu Gln /	Asp Glu Val Val Asp
	100	105	110
Glu Val Val	Ser Asp Thr Ala (Ju Ala Pro Lys S	Ser Ala Glu Glu Gln
115		120	125
Ala Ala Glu	Ser Ala Lvs Pro F	tis Ivs Val Ive H	his Arg Thr Phe His
130	135		
Tyr Pm Gly		1	.40
145	ory Leu var ASP F	ne val Lys His I	le Asn Arg Thr Lys
	150	155	160
ASII Pro 11e	Gin Gin Ser Vai I	1e Asp Phe Asp G	ly Lys Gly Thr Gly
	165	170	175
His Glu Val	Glu Ile Ala Met G	In Trp Asn Glv G	ly Tyr Sor Clu Son
	180	185	
			190
Val His Thr	Phe Ala Asn The T	lo Aca Than is an	
105	Phe Ala Asn Thr I	ie Asn inr His Gi	lu Gly Gly Thr His
יינים יין כויי)O	205
old Gld Gly	Phe Arg Ser Ala Le	ou Thr Ser Val Va	ll Asn Lys Tyr Ala
210	215	22	0
Lys Asp Lys	Lys Leu Leu Lys As	p Lys Asp Pro As	n Leu Thr Gly Asn
225	230	235	
Asp Ile Arg	Glu Gly Leu Ala Al	a Val Tlo Son Va	240
.,	245		r Lys var Ala Glu
Pro Cin Pho (250	255
THE CITY FIRE C	Glu Gly Gln Thr Ly	s Thr Lys Leu Gly	/ Asn Thr Glu Val
	260	265	270
Lys Ser Phe \	al Gln Lys Val Cy	s Asn Glu Gln Leu	Thr His Tro Phe
2/5	280)	285
Glu Ala Asn F	ro Ser Glu Ala Lys	Thr Val Val Asn	live Ala Val Cam
290	295		
Ser Ala Gln A		300	
305	la Arg Ile Ala Ala	LARG LYS ATA ARG	Glu Leu Val Arg
	310	315	320
Arg Lys Ser A	la Thr Asp Leu Gly	'Gly Leu Pro Gly	Lys Leu Ala Asp
	325	330	775
Cys Arg Ser T	hr Asp Pro Arg Lys	Ser Glu Leu Tvr	Val Val Clu Clu
34	40	345	
			350
355	ly Gly Ser Ala Lys	ser diy Arg Asp	ser Met Phe Gln
	360		365
Ald Tie Fen bi	o Leu Arg Gly Lys	Ile Ile Asn Val	Glu Lys Ala Arg
370	375	380	••
Ile Asp Arg Va	I Leu Lys Asn Thr	Clu Val Cla Ala	77 - 77

390

395

400

72

Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr

His Lys Ile Val Leu

420

<210> 24

<211> 419

<212> PRT

<213> Mycobacterium bovis

<400> 24

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Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Glu Ile Lys Arg Asp 20 25 30

Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu 35 40 45

Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp
50 55 60

Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val
65 70 75 80

Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile 85 90 95

Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val
100 105 110

Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala 115 120 125

Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro 130 135 140

Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala

Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu
165 170 175

Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 180 185 190

Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu 195 200 205

Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp 210 215 220

Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile 225 230 235 240

Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln 245 250 255

Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser 260 265 270

Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 275 280 285

Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala 290 295 300

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Gìn Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys
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                                            315
    Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg
                                        330
    Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser
                                    345
    Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile
                                360
    Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp
                           375
   Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly
                       390
                                           395
   Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys
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                                       410
   Ile Val Leu
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  Arg Asp Gly His Lys Trp Ser Gln Phe Tyr Asn Lys Ala Val Pro Gly
                               40
  The Leu Lys Gln Gly Glu-Ala The Lys Lys The Gly The The Ile Arg
                           55
  Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
                       70
                                          75
 Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                                      90
 Thr Ile Asn Leu Thr Asp Glu Arg Val Ala Gln Asp Glu Val Val Asp
                                 105
 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys
                             120
                                                 125
Ala Ala Glu Ser Lys Gly Pro His Lys Val Lys His Arg Thr Phe His
                         1.35
                                             140
Tyr Pro Gly Gly Leu Ile Asp Phe Val Lys His Ile Asn Arg Thr Lys
                    150
Ser Pro Ile Gin Gin Ser Val Val Ala Phe Asp Gly Lys Gly Glu Gly
                165
                                    170
His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                            200
                                                205
Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
                        215
Lys Asp Lys Lys Leu Leu Lys Glu Lys Asp Ala Asn Leu Thr Gly Asp
                    230
                                        235
                                                           240
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Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
 Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
                                 265
Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                            280
Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser
                        295
                                            300
Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                         315
Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                                    330
Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                345
Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
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Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                        375
Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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His Lys Ile Val Leu
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Thr Leu Lys Gln Gly Glu Ala Thr Lys Thr Thr Gly Thr Thr Ile Arg
                         55
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
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Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
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Thr Ile Asn Leu Thr Asp Glu Arg Val Ser Glu Glu Glu Val Val Asp
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Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Val Glu Lys
                            120
Ala Ala Glu Ser Thr Gly Pro His Lys Val Lys His Arg Thr Phe His
                        135
                                            140
Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
                    150
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Asn Pro Ile His Asn Ser Ile Val Asp Phe Ser Gly Lys Gly Pro Gly

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165
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   Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
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   Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
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                                              220
   Lys Asp Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
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                                          235
   Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu
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   Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
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   Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                              280
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  Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                         315
  Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
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                                     330
  Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
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  Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
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 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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 His Lys Ile Val Leu
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                             40
Thr Leu Lys Gln Gly Glu Ala Thr Lys Arg Thr Gly Thr Thr Ile Arg
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
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Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
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                                    90
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80
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Thr	He	Asn	Leu 100		Asp	Glu	Arg	Va1 105		Asn	Glu	Glu			Asp
Glu	Val	۷a٦			Thr	Ala	Asp			Lys	Ser	Αla	110 Gln		Lys
		115					120					125			·
Ala	A7a 130		Ser	Thr	Ala	Pro 135	His	Lys	Val	Lys	His 140	Arg	Thr	Phe	His
Tyr	Pro	Gly	Gly	Leu	Va1	Asp	Phe	Val	Lys	His	Ile	Asn	Arg	Thr	Lys
145					150					155					160
Ser	Pro	Ile	Gln	G]n 165		IJe	Ile	Asp	Phe 170		Gly	Lys	Gly	Pro 175	Пy
His	Glu	۷a٦	Glu 180	Пe	Ala	Met	Gln	Trp 185	Asn	Gly	G٦y	Tyr	Ser 190	Glu	Ser
Val	His	Thr 195	Phe	Ala	Asn	Thr	Ile 200	Asn	Thr	His	Glu	G7y 205		Thr	His
Glu	Glu		Phe	Arg	Ser	Ala	Leu	Thr	Ser	۷a٦	Val		Lys	Tyr	Ala
	210					215					220		-	·	
Lys	Asp	Lys	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu	Thr	G٦y	Asp
225					230					235					240
Asp	He	Arg	Glu		Leu	Ala	Ala	Val		Ser	Val	Lys	۷a۱		Glu
Dro	Cln	Dha	cı	245	C]n	The	Lvc	Th.	250		C1	A =	Th	255	\
FIU	GIII	riie	260	uly	GIII	HH	Lys	265	LyS	Leu	GIY	ASn	270	Giu	vai
Lvs	Ser	Phe		Gln	Lvs	۷a۱	Cys		Glu	Gln	Leu	Thr		Trn	Phe
•		275			-,-		280			•		285	5		1110
۵lu	Ala	Asn	Pro	Ala	Asp	Аlа	Lys	Val	Va1	۷a٦	Asn	Lys	Ala	Val	Ser
	290					295					300				
Ser	Ala	Gln	Ala	Arg	Пe	A٦a	Ala	Arg	Lys	Ala	Arg	Glu	Leu	Val	Arg
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				325			Gly		330					335	
Cys	Arg	Ser	Thr 340	Asp	Pro	Arg	Lys	Ser 345	Glu	Leu	Tyr	Val	Va1 350	Glu	σIу
Asp	Ser	A1a 355	Gly	GTy	Ser	Ala	Lys 360	Ser	Gly	Arg	Asp	Ser 365	Met	Phe	Gln
Ala	Ile	Leu	Pro	Leu	Arg	G٦y	Lys	Ile	Пe	Asn	Val	GTu	Lys	Ala	Arg
	370					375					380				
Ile 385	Asp	Arg	Val	Leu	Lys 390	Asn	Thr	Glu	Val	G]n 395	Ala	Ile	Ile	Thr	A1a 400
	Gly	Thr	GJV	Пe		Asp	G1u	Phe	Asp		Thr	Lvs	l eu	Ara	
				405					410			-,-		415	.,.
His	Lys	Ile	Va1 420	Leu											
<210	> 28														
	> 42														
	> PR														
	Ī		cter	'i um	aviu	m									
	> 28		Ca-	C 1	T	A ===	V-7	c -	~ 1	~			6 3		~ 3
υίγ 1	oiu .	ASI	ser	G 19 5	ıyr	ASN	Val	ser		uly	reu	HIS	OIV.		GIY
	Ser	۷a۱ ٔ	Val.	_	ΑΊa	Leu	Ser	Thr	1.0 Ara	Leu	Glu '	Val	Asn	15 Tle	Αla
															* * * * * *

20	25	30
Arg Asp Gly Tyr Glu Trp Se	r Gln Tyr Tyr ,	ASD His Ala Val Pro Cly
35	40	45
Thr Leu Lys Gln Gly Glu Ala	Thr Lys Ara	The Gly The The Ilo Are
50 55	i	60
Phe Trp Ala Asp Pro Asp Ile 65 70	Phe Glu Thr 1	75
Thr Val Ala Arg Arg Leu Gln	Glu Met Ala f	75 80 The Leu Asn Lys GTy Leu
85	90	95
Thr Ile Asn Leu Thr Asp Glu 100	Arg Val Thr A	
Glu Val Val Ser Asp Thr Ala		110 Ys Ser Ala Gln Glu Ivs
115	120	125
Ala Ala Glu Ser Ala Ala Pro	His Lys Val L	VS His Ara Thr Pho Hic
130 135		140
Tyr Pro Gly Gly Leu Val Asp	Phe Val Lvs H	
145 150		
Asn Pro Ile His Gln Ser Ile		
165	170	
His Glu Val Glu Ile Ala Met		175
180	185	
Val His Thr Phe Ala Asn Thr		190
	200	
Glu Glu Gly Phe Arg Ser Ala		205
210 215	ced IIII Ser va	
Lys Asp Lys Lys Leu Leu Lys /	Ace luc Ace O.	220
4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		5
Asp Ile Arg Glu Gly Leu Ala A 245		r Val Lys Val Ser Glu
	250	255
Pro Gln Phe Glu Gly Gln Thr L 260		J Gly Asn Thr Glu Val
	265	270
Lys Ser Phe Val Gln Lys Val C		Leu Thr His Trp Phe
	80	285
Glu Ala Asn Pro Ala Asp Ala L	ys Val Ile Val	Asn Lys Ala Val Ser
290 295		300
Ser Ala Gln Ala Arg Ile Ala A	la Arg Lys Ala	Arg Glu Leu Val Arg
305 310	315	320
Arg Lys Ser Ala Thr Asp Leu G	ly Gly Leu Pro	Gly Lys Leu Ala Asp
325	330	335
Cys Arg Ser Thr Asp Pro Arg Ly	s Ser Glu Leu	Tyr Val Val Glu Glv
340	345	350
Asp Ser Ala Gly Gly Ser Ala Ly	s Ser Glv Ara	Asn Ser Met Phe Cln
355 . 36		365
Ala Ile Leu Pro Leu Arg Gly Ly	'S Ile Ile Asn	
370 375	210 7611	380
Ile Asp Arg Val Leu Lys Asn Th	r Glu Vəl Glə	
385 390		
550	395 U Pho Acn Tla	400
Leu Gly Thr Gly Ile His Asp Gl 405		
His Lys Ile Val Leu	410	415

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<211> 421

<212> PRT

<213> Mycobacterium gordonae

<400> 29

Gly Glu Asn Ser Gly Tyr Thr Val Ser Gly Gly Leu His Gly Val Gly

1 5 10 15 /al Ser Val Val Asp Ala Leu Ser Thr Arg Leu Glu Val Asp Tle Lv

Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys
20 25 30

Arg Asp Gly His Glu Trp Ser Gln Tyr Tyr Lys Arg Ala Val Pro Gly
35 40 45

Thr Leu Lys Gln Gly Glu Thr Thr Arg Lys Thr Gly Thr Thr Ile Arg
50 55 60

Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu
65 70 75 80

Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu 85 90 95

Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp
100 105 110

Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys 115 120 125

Ala Ala Glu Ser Lys Ala Pro His Lys Val Lys Gln Arg Thr Phe His 130 135 140

Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys

Ser Pro Ile Gln Gln Ser Val Ile Asp Phe Glu Gly Lys Gly Thr Gly
165 170 175

His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 185 190

Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glú Gly Gly Thr His 195 200 205

Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 215 220

Lys Asp Lys Lys Leu Leu Lys Glu Lys Asp Pro Asn Leu Thr Gly Asp 225 230 235 240

Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
245 250 255

Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val 260 265 270

Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285

Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser 290 295 300

Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg 305 310 315 320

Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 335

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26
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Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                   345
   Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
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   Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                          375
   Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
                      390
                                          395
  Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr
                  405
                                      410
  His Lys Ile Val Leu
  <210⊳ 30
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 Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu
                              40
 Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp
      50
                          55
 Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val
  65 _____70__
                                 - - . - . 75
 Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile
                                     90
 Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val
                                105
 Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala
                            120
 Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro
                        135
                                            140
Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala
                                        155
Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu
                165
                                    170
Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His
Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu
                            200
                                               205
Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp
                       215
Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile
                   230
                                       235
Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln
                                   250
Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser
```

260 265 270 Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 280 Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala 295 300 Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys 315 Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg 330 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 360 Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 375 Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly 390 395 Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys 405 410 Ile Val Leu <210> 31 <211> 419 <212> PRT <213> Mycobacterium tuberculosis <400> 31 Ser Asp Ala Tyr Ala Ile Ser Gly Gly Leu His Gly Val Gly Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Glu Ile Lys Arg Asp 25 Gly Tyr Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Leu Gly Leu 40 Lys Gln Gly Ala Pro Thr Lys Lys Thr Gly Ser Thr Val Arg Phe Trp 55 Ala Asp Pro Ala Val Phe Glu Thr Thr Glu Tyr Asp Phe Glu Thr Val 70 75 Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu Thr Ile 90 Asn Leu Thr Asp Glu Arg Val Thr Gln Asp Glu Val Val Asp Glu Val 100 105 Val Ser Asp Val Ala Glu Ala Pro Lys Ser Ala Ser Glu Arg Ala Ala 120 125 Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His Tyr Pro 135 140 Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn Ala 155 Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 165 170 Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185

Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu

195	200	205
Gly Phe Arg Ser Ala Leu	Thr Ser Val Val	
210	215	220
Arg Lys Leu Leu Lys Asp	Lys Asp Pro Asn	
225 230		235 240
Arg Glu Gly Leu Ala Ala	Val Ile Ser Val	
245	250	255
Phe Glu Gly Gln Thr Lys		
260	265	
Phe Val Gln Lys Val Cys		270
275	280	
Asn Pro Thr Asp Ala Lys		285
200	295	
Gln Ala Arg Ile Ala Ala		300
305 310		~-
310		315 320
Ser Ala Thr Asp Ile Gly (325		
	330	335
Ser Thr Asp Pro Arg Lys 5 340		
	345	350
Ala Gly Gly Ser Ala Lys S 355		
	360	365
Leu Pro Leu Arg Gly Lys I 370	75	
•		380
Arg Val Leu Lys Asn Thr G 385 390	•	
330		95 400
Thr Gly Ile His Asp Glu P		
Ile Val Leu	410	415
<210> 32		
<211> 419		
<212> PRT		
<213> Mycobacterium gastr	i	
<400> 32		
	on Clu Clu I am Is	
Ser Asp Ala Tyr Ala Ile Se		
<u>-</u>	10	1.7
Val Val Asn Ala Leu Ser Il 20		al Glu Ile Lys Arg Asp
	25	. 30
Gly His Glu Trp Ser Gln Va		er Glu Pro Met Gly Leu
35	40	45
Lys Gln Gly Ala Pro Thr Ly		ir Thr Val Arg Phe Trp
	5	60
Ala Asp Pro Asn Val Phe Gl		
65 70	7	- 00
Ala Arg Arg Leu Gln Glu Me	t Ala Phe Leu As	n Lys Gly Leu Thr Ile
85	90	95
Asn Leu Thr Asp Gln Arg Va	l Thr Gin Asp Gi	u Val Val Asp Glu Val
100	105	110
Val Ser Asp Val Ala Glu Ala		a Ser Glu Lys Ala Ala
115	120	125
Glu Phe Thr Ala Pro His Lys	Val Lys Lys Arc	Thr Phe His Tvr Pro

(47)91 · 92 130 135 Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arq Thr Lys Asn Ala 150 155 Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 165 170 Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185 Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu 200 Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile 230 235 Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln 250 Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser 265 Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 280 Asn Pro Ala Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser Ser Ala 295 300 Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg 330 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser

Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 360

Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 375

Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly 390 395

Thr Gly Ile His Asp Glu Phe Asp Ile Ala Arg Leu Arg Tyr His Lys 405 410

Ile Val Leu

<210> 33

<211> 421

<212> PRT

<213> Mycobacterium marinum

<400> 33

Gly Glu Asn Ser Gly Tyr Asn Val Ser Gly Gly Leu His Gly Val Gly 10

Val Ser Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Asp Ile Lys

Arg Asp Gly Tyr Glu Trp Ser Gln Phe Tyr Asp Arg Ala Gln Pro Gly 40

Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg

Phe Trp Ala Asp Ser Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu

<213> Mycobacterium microti

<400> 34

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65
                       70
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  Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Pro Asp Glu Val Val Asp
                                  105
  Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Gln Glu Lys
                             120
  Ala Ala Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His
                         135
  Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
                     150
                                         155
 Ser Pro Ile Gln Gln Ser Ile Val Asp Phe Glu Gly Lys Gly Ser Gly
                                     170
 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
                                 185
 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                             200
 Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
 Lys Asp Lys Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
                     230
                                        235
 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Arg Val Ala Glu
                 245
 Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
                                265
 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
       275
Glu Ala Asn Pro Ser Glu Ala Lys Thr Ile Val Asn Lys Ala Val Ser
Ser Ala Gln Ala Arg Leu Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                        315
Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                                    330
Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
            340
Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                            360
Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                       375
Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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Leu Gly Thr Gly Ile His Asp Glu Phe Asp Leu Ser Lys Leu Arg Tyr
                                   410
                                                       415
His Lys Ile Val Leu
           420
<210> 34
<211> 419
<212> PRT
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ч	n

Ser	Asp	Ala	Tyr	Ala	Ile	Ser	· GTy	/ GTy	/ Leu	His	Gly	Val	Gly	′ Va1	Ser
1				5					10					15	
Val	Val	Asn	A1a 20		Ser	Thr	Arc	Leu 25		Val	Glu	Ile	: Lys 30		Asp
σIу	Tyr	G]u 35	Trp	Ser	Gln	Val	Tyr 40		Lys	Ser	Glu	Pro		Gly	Leu
Lys	G1n 50		Ala	Pro	Thr	Lys 55		Thr	· GTy	Ser	Thr 60			Phe	Trp
Ala			Ala	Va1	Phe			Thr	· Glu	Tvr		Phe	Glu	Thr	Va1
65			,,,,		70		••••		0.0	75	ΛOP	1110	- Giu	,,,,,	80
A٦a	Arg	Arg	Leu	Gln	Glu	Met	Αla	Phe	Leu	Asn	Lys	G٦y	Leu	Thr	Пe
				85					90					95	
Asn	Leu	Thr	Asp 100	Glu	Arg	Val	Thr	G]n 105		Glu	Val	Val	Asp 110	Glu	Va1
۷a٦	Ser	Asp 115	Va1	Аlа	Glu	Ala	Pro 120		Ser	Ala	Ser	GTu 125	Arg	Ala	Аlа
Glu	Ser 130	Thr	Аlа	Pro	His	Lys 135	۷a۱	Lys	Ser	Arg	Thr 140	Phe	His	Tyr	Pro
GΙγ	Gly	Leu	Va1	Asp	Phe	۷a٦	Lys	His	Ile	Asn	Arg	Thr	Lys	Asn	Αla
145					150					155					160
Ile	His	Ser	Ser	Ile	Val	Asp	Phe	Ser	Gly	Lys	Gly	Thr	G٦y	His	Glu
			_ (165					170					175	
Val	Glu	Ile	Ala 180	Met	GIn	Trp	Asn	A7a 185	Gly	Tyr	Ser	Glu	Ser 190	Val	His
Thr	Phe	Ala 195	Asn	Thr	Ile	Asn	Thr 200	His	GTu	Πy	Gly	Thr 205	His	Glu	Glu
GΊγ	Phe 210	Arg	Ser	Ala	Leu	Thr 215	Ser	۷aΊ	Val	Asn	Lys 220	Tyr	Ala	Lys	Asp
Arg	Lys	Leu	Leu	Lys	Asp	Lys	Asp	Pro	Asn	Leu		GΊγ	Asp	Asp	Пe
225					230					235					240
Arg	Glu	GΊγ	Leu	A1a 245	Ala	Val	Ile	Ser	Va1 250	Lys	Val	Ser	Glu	Pro 255	۵In
Phe	Glu	Gly	Gገո 260	Thr	Lys	Thr	Lys	Leu 265	Gly	Asn	Thr	Glu	Va1 270	Lys	Ser
Phe	Val	G1n 275	Lys	Val	Cys	Asn	G1u 280		Leu	Thr				Glu	Ala
4sn			Asp	Ser				Val	Asn	Lys	Ala	285 Va1	Ser	Ser	Ala
:In	290 Ala	Ara	Ile	Δla		295 Am	Lvc	Δla	Ara	СIII	300 Lau	\/ - 1	۸ra	Ara	Lve
305	,	,,			310	A1 94	Lys	AIG	лія	315	LCJ	Vai	AI Y	Al y	320
Ser	Ala	Thr	Asp		GJ y	G٦y	Leu	Pro		-	l.eu	Ala	Asp		
ser	Thr	Asp	Pro .	325 Ara	Lvs	Ser	Glu	Leu	330 Tvr	Va1	Va1	Glu	Glv	335 Asp	Ser
			340					345					350		
Ala	GΊγ	G7y 355	Ser .	A1a	Lys	Ser	G1γ 360	Arg	Asp	Ser		Phe 365	G1n	Ala	IJe
.eu	Pro 370	Leu .	Arg (Gly		Ile 375	Ile	Asn	Val		Lys . 380	Ala	Arg	Ile	Asp
۱rg		Leu	Lys .	Asn '			Val	Gln	Ala			Thr	Ala	Leu	IJУ
385					390					395					400

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                                       410
   Ile Val Leu
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                                       10
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  Arg Asp Gly His Glu Trp Ser Gln Tyr Tyr Glu Arg Ala Val Pro Gly
  Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
                           55
  Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu
                      70
  Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                  85
                                       90
  Thr Ile Asn Leu Thr Asp Glu Arg Val Asp Gln Asp Glu Val Val Asp
             100
                                 105
 Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Glu Glu Lys
                             120
 Ala Ala Glu Ser Lys Ala Pro His Lys Val Lys His Arg Thr Phe His
                         135
 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys-His Fle-Asn Arg Thr Lys-
 145
                     150
 Ser Pro Ile Gln Gln Ser Val Ile Asp Phe Glu Gly Lys Gly Thr Gly
                 165
                                     170
 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
                                 185
Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                             200
                                                 205
Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
                        215
Lys Asp Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
                    230
                                         235
Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
                245
Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
                                265
Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
        275
                            280
Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser
                        295
                                            300
Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                        315
Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                325
                                    330
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. 100

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Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
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 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                             360
 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                        375
 Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
                    390
                                        395
 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Ser Lys Leu Arg Tyr
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 His Lys Ile Val Leu
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Arg Asp Gly His Glu Trp Phe Gln Tyr Tyr Asp Arg Ala Val Pro Gly
                             40
Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg
Phe Trp Ala Asp Pro Glu Ile Phe Glu Thr Thr Gln Tyr Asp Phe Glu
                   70
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
                                     90
Thr Ile Asn Leu Thr Asp Glu Arg Val Glu Gln Asp Glu Val Val Asp
                                105
Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys
                            120
Ala Ala Glu Ser Thr Ala Pro His Lys Val Lys His Arg Thr Phe His
                        135
Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys
                    150
Ser Pro Ile Gln Gln Ser Val Ile Asp Phe Asp Gly Lys Gly Thr Gly
                165
                                    170
His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
                                185
Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
                           200
                                               205
Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
                        215
                                           220
Lys Asp Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
                   230
                                       235
Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ala Glu
                                   250
Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
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260
                                   265
  Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                               280
  Glu Ala Asn Pro Ser Glu Ala Lys Thr Val Val Asn Lys Ala Val Ser
                          295
                                              300
  Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                      310
                                          315
  Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp
                                      330
  Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
                                  345
  Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln
                                                  365
 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                          375
 Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr
                                      410
 His Lys Ile Val Leu
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Thr Asp Gly Tyr Glu Trp Phe Gln His Tyr Asp Arg Ser Val Pro Gly
Thr Leu Lys Gln Gly Glu Lys Thr Lys Lys Thr Gly Thr Thr Val Arg
                         55
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Asp Tyr Asp Phe Glu
                     70
                                       : 75
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
Thr Ile Asn Leu Thr Asp Glu Arg Val Arg Asn Glu Glu Val Val Asp
Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Arg Glu Glu
                            120
Ala Glu Glu Arg Thr Thr Gln Lys Val Lys His Arg Thr Phe His Tyr
                        135
Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys Asn
                                       155
Pro Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Pro Gly His
                                   170
Glu Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val
           180
                                185
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His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu
                             200
 Glu Gly Phe Arg Ala Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys
                         215
 Asp Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp
                     230
                                         235
 Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro
                                    250
 Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys
                                265
 Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu
                             280
 Ala Asn Pro Ser Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser Ser
                         295
Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg
                     310
 Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys
                325
                                    330
Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp
                                345
Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala
                            360
Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile
                         375
Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu
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Cly Thr Gly Ile His Asp Glu Phe Asp Ile Ser Lys Leu Arg Tyr His
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Lys Ile Val Leu
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Arg Asp Gly Tyr Glu Trp Ser Gln Tyr Tyr Asp His Ala Val Pro Gly
                             40
Thr Leu Lys Gln Gly Glu Ala Thr Lys Arg Thr Gly Thr Thr Ile Arg
                         55
Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu
                     70
Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu
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Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Asn Glu Glu Val Val Asp
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Glu Val Val Ser Asp Thr Ala Asp Ala Pro Lys Ser Ala Gln Glu Lys
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115
                              120
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  Asn Pro Ile His Gln Ser Ile Ile Asp Phe Gly Gly Lys Gly Pro Gly
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  His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser
              180
                                 185
  Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His
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  Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala
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 Lys Asp Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp
 225
                     230
                                         235
 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu
                                     250
 Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val
 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe
                             280
 Glu Ala Asn Pro Ala Asp Ala Lys Val Ile Val Asn Lys Ala Val Ser
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 Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg
                                        315
 Arg Lys Ser Ala Thr Asp Leu Cly Cly Leu Pro Gly Lys Leu Ala Asp
                325
 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly
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                                345
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Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg
                        375
Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala
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                                        395
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His Lys Ile Val Leu
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ctgcgttcgt atatgagcnc crtcnacrtc ngcrtc

【図面の簡単な説明】

*を用いたPCRの結果を示す図。

【図1】マイコバクテリウム・カンサシイとマイコバク テリウム・ガストリのgyrB遺伝子の塩基配列を示す図。

【図3】分子系統解析により作製された遅発育性マイコ バクテリアの系統樹を示す図。

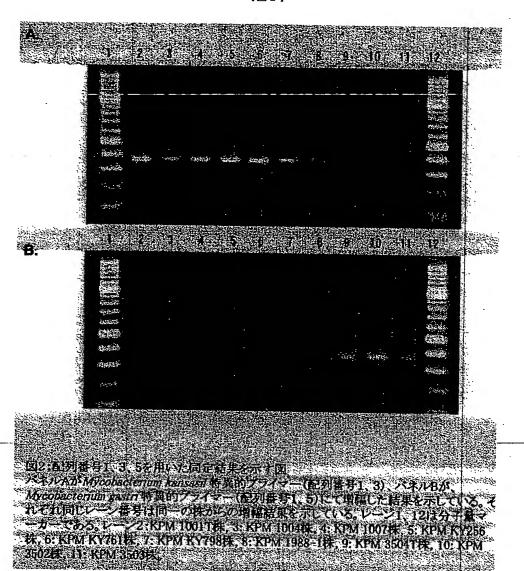
【図2】配列番号1、3、5により表されるプライマー*

【図1】

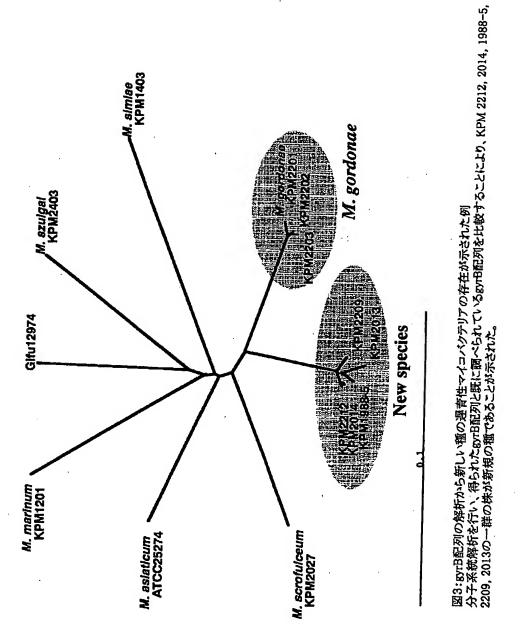
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HEAR HEAS	61:CTCTOCACCOCCTCCACCTTCCACATCACCCCCACCCCA
HEADI	131:GACAAATCOCAGCOCATGGGACTCAAGCAACCACCCCCCCACACG 180 181:
MEAS MEAS	181:GIDOGETICTGGGCGAPCOCAATCTTTTGGAGCACGGGTAGGACTTGGAAACOSTC 240 181:
MEAN MGAS	241:GCACGAOGGTTGCAGGACATGGCGTTTCTCAACAAGGGCCCACCATCAATCTGACCGAT 200 241:G
WAN Was	201:CACCCCCTCACCTACACCAGETCETCACCACCTGCTCACCACGTGCCCCCACCACCGCGCCCCCCCC
WEAR NGAS	261: MGTOCGCCMCTAEAMOCCGCCCAATCCCCCGCCCCCCCCCAAAGCCTCACAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCTCACCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCCAAGCAAAGCCAAGCCAAGCA
MICAN MGAS	421:TICLACTATOLLEGGGGTGTGCACTTGCACTGGACGCACAACGCCACGAACGC 479 421:TTCT
MGAS	480: CATOCACACCACCATOGTOLACTICTOCOCTAACCCACCOCOCTACCAAGTOCACATCE 8.38 480:
MGAS	630:CBARCCAETGCAATGCCCCCTATTCCGAGTGCGTGCATACCTTGCCCAACACCATCAACA 898 630:
YEAN WGAS	E99::CCLACAGGGGGGACCAGGAGGAGGGGTTCCCCAGGCGCGCCCCACCTCGGTGGTGAACA 658 E99:T
NECAS NECAS	659: ACTRODOCARGEACOCCAARCTECTCARGEALARGEACOCCAACCTCRODOCACGACA 718 659:
MEAN MGAS	719: TOCGGLAGGGTTGGCCCCCCCCCCCCCCCCCCCCCCCCCCCC
NGAS	779: AGRICIANGROGALACTOCOCHICIACOGAGGTGAACTOCTTGCTGCAGAACGTGTGCAACG 823 779:
ncan Ngas	E39:.ACCCTCCCCATTCGTTCLAGGCCAACCCTCAACCCTTGTCAACCAGO 898 E39:
HEAS	899: A
ncas NCAS	958:AAGACGCCAACGATCTGGCCGGATTACXIDGCAAGCTGCCCGACCTGCACCGACCGACCGACCGACCGAC
NGAS	1918:CCGCCCAGGCCAACGCGAGGCGGAGGCGAGGCCGATTCACCCCCCCAACACCC (677 1918:C.T
	1078: COTCOCEACTOCATETROCASSOCATOTISCOCTISCOCCECAACATCATCATCATCTCASC 1127 1078: T. A. C
NGAS	1128: AAGGOOOGGA TOGACOGGGTGAAGAACAOGGAAGTOCAGGGGATCATCAOCGGGTTG 1197 1128:
WAN NGAS	1198:-GCTACCCCCATCCACCACCACTCCCATCCCCCCCCCCCC
图1: 同一的	Bycobacterius kansasii(上限)とBycobacterius gastri(下限)の gyrb配列の比較。 概据配列の部分は長印で示し、Bycobacterius kansasii の配列のみを示した。

【図2】



【図3】



フロントページの続き

(72)発明者 原山 重明

岩手県釜石市平田第3地割75番1 株式会 社海洋バイオテクノロジー研究所釜石研究 所内 Fターム(参考) 4B024 AA11 CA09 DA05 HA19 4B063 QA01 QQ06 QQ42 QR32 QR55 QR62 QS25

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The 16s/23s ribosomal spacer region as a target for DNA probes to identify eubacteria.

Barry T, Colleran G, Glennon M, Dunican LK, Gannon F.

National Diagnostic Centre, BioResearch Ireland, University College Galway.

Variable regions of the 16s ribosomal RNA have been frequently used as the target for DNA probes to identify microorganisms. In some situations, however, there is very little sequence variation observed between the 16s rRNA genes of closely related microorganisms. This study presents a general method to obtain species-specific probes using the spacer (intergenic) region between the 16s and 23s rRNA genes. The overall strategy is analogous to that which has previously been developed for the variable regions of the 16s rRNA genes. Sequence analysis of the 16s rRNA and 23s rRNA coding sequences flanking the spacer regions resulted in the design of PCR primers that can be used to amplify the spacer regions of a wide range of eubacteria. Sequencing the amplified spacer region then gives rise to the information that can be used to select specific DNA sequences for use as a DNA probe or for the generation of specific PCR primers to a microorganism of interest. In this study the approach to develop specific DNA markers for members of the genus Clostridium is described in detail. A specific DNA oligonucleotide probe and PCR primers have been designed for Clostridium perfringens that distinguish it from other organisms in the genus.

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